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*PATHWAY OF SERINE FORMATION FROM CARBOHYDRATE IN RAT LIVER**

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Communicated by H. A. Barker, June 8, 1955

There are a number of reports on the formation of serine from glucose in animal tissues,[1–3] but experimental evidence for the steps in the main pathway of serine formation has not been available up to now. Sallach[4] has recently reported the presence of a transaminase between 3-hydroxypyruvic acid and alanine and has presented a possible pathway of serine formation from D-glyceric acid through 3-hydroxypyruvic acid. This paper reports the formation of serine from glucose, D-glyceric acid, 3-phosphoglyceric acid, and 3-phosphohydroxypyruvic acid by a rat-liver enzyme system and presents an alternative pathway of serine formation.

EXPERIMENTAL

Preparation of Enzyme System.—The livers from male rats, weighing 250–300 gm., were homogenized with 2 volumes of 0.1 *M* phosphate buffer (pH 7.4) in a Waring Blendor for 40 seconds. The homogenate was then centrifuged at 600 × g for 10 minutes and the supernatant fluid recentrifuged at 105,400 × g for 30 minutes in the Spinco preparative ultracentrifuge. The supernatant fluid from this was fractionated with saturated $(NH_4)_2SO_4$, and the enzyme activity was found in the fraction precipitating between 33–66 per cent saturation. This was dissolved in 0.1 *M* phosphate buffer and dialyzed against 0.03 *M* phosphate buffer, both at pH 7.4, for 6 hours. This served as the enzyme system. When inorganic phosphate determination was required, 0.2 *M* Tris buffer (pH 7.4) was substituted. All isolation procedures were carried out in the cold (<3° C.).

Radioactive DL-glyceric acid was purchased from Isotopes Specialties Co., Glendale, California, and pyruvamide and zinc lactate from Tracerlab, Inc. Radioactive glucose was generously furnished by Dr. W. Z. Hassid, radioactive 3-phosphoglyceric acid by Dr. J. A. Basham, and unlabeled 3-phosphohydroxypyruvic acid by Dr. C. E. Ballou.[5]

Assay Methods.—One milliliter of the enzyme preparation, the indicated substrates and cofactors, and 2 ml. of buffer solution in a total volume of 5 ml. were incubated for 2 hours at 37° C. in Warburg vessels. The reaction was stopped by the addition of trichloroacetic acid to give a final concentration of 10 per cent.

When radioactive substrates were employed, the serine formed was determined by adding carrier serine and recrystallizing to constant specific activity according

to the method of Siekevitz and Greenberg.[6] The serine formed from nonradioactive substrates was determined by periodate oxidation and measurement of the formaldehyde, according to the method of Frisell *et al.*[7] Inorganic phosphate was measured by the method of Lowry and Lopez.[8]

RESULTS

Serine Formation from Uniformly Labeled Glucose.—Table 1 shows the formation of serine from uniformly labeled glucose and the considerable dilution of the radioactivity in the serine by the addition of nonradioactive 3-phosphoglyceric acid. The highest results were obtained anaerobically and in the presence of NaF, which would preclude the reaction proceeding to pyruvic acid. The tissue with the highest activity of serine formation was liver (Table 2).

TABLE 1

SERINE FORMATION FROM UNIFORMLY LABELED GLUCOSE INCUBATIONS WITH 66.8×10^4 C.P.M. OF RADIOGLUCOSE

Atmosphere	Addition	Serine (c.p.m.)	Respiratory CO_2 (c.p.m.)
O_2	None	360	255
N_2	None	560	75
O_2	5×10^{-3} *M* NaF	160	138
N_2	5×10^{-3} *M* NaF	960	68
N_2	NaF, 200 μM PGA*	313	...

* PGA = Nonradioactive 3-phosphoglyceric acid.

TABLE 2

ACTIVITY OF SERINE FORMATION OF VARIOUS TISSUES*

Tissue	Serine (c.p.m.)
Liver	1,740
Kidney	460
Spleen	0

* Each flask contained 66.8×10^4 c.p.m. of radioglucose, 400 mg. of tissue homogenate, 5×10^{-3} *M* NaF, and 2 ml. of 0.1 *M* phosphate buffer (pH 7.4) in 5 ml. anaerobic incubation.

Serine Formation from Pyruvic Acid-2-C^{14} and Lactic Acid-2-C^{14}.—Although it has been reported that serine was synthesized from pyruvic acid,[9] neither pyruvamide-2-C^{14} (16×10^5 c.p.m.) nor lactic acid-2-C^{14} (46×10^5 c.p.m.) yielded any appreciable serine with our enzyme, and this negative result was not altered by addition of either ATP, KCl, or DPN.

Serine Formation from Uniformly Labeled and Nonradioactive 3-Phosphoglyceric Acid.—The dilution of the radioactive serine by nonradioactive 3-phosphoglyceric acid in the presence of NaF (Table 1) suggested that serine might be formed from 3-phosphoglyceric acid without passing through pyruvic acid. This possibility was tested with both uniformly labeled and nonradioactive 3-phosphoglyceric acid. It was found that 3-phosphoglyceric acid was converted to serine and, moreover, that the reaction was accelerated by DPN and alanine or glutamic acid (Tables 3 and 4). No difference was found in the stimulation of serine formation by these two amino acids in these crude enzyme preparations.

Serine Formation from D-*Glyceric Acid-3-C^{14}.*—Although it was reported that D-glyceric-1-C^{14} acid gives considerable amounts of serine,[4] it was found that the

conversion of D-glyceric acid to serine was only slight without ATP but was significant in the presence of ATP, DPN, and alanine (Table 5).

TABLE 3

SERINE FORMATION FROM UNIFORMLY LABELED 3-PHOSPHOGLYCERIC ACID*

Additions	Serine (c.p.m.)	Recovery (Per Cent)
None	10,253	2.0
Glutamate (20 μM)	25,628	5.2
DPN (400 μgm.)	19,254	3.8
ATP (20 μM)	18,434	3.6
Glutamate, DPN	33,238	6.6
Glutamate, DPN, ATP	33,596	6.7

* Anaerobic incubations with 5×10^5 c.p.m. of uniformly labeled 3-phosphoglyceric acid and 5×10^{-3} *M* NaF.

TABLE 4

SERINE FORMATION FROM NONRADIOACTIVE 3-PHOSPHOGLYCERIC AND 3-PHOSPHOHYDROXY PYRUVIC ACID

Additions	Serine (μM)	Inorganic P (μM)	Recovery (Per Cent)
PGA (5 μM)*, †	0	0.5	0
PGA, DPN (400 μgm.)	0.52	...	10.4
PGA, DPN, alanine (7.5 μM)†	0.66	1.37	13.2
PGA, DPN, alanine, NaF (5×10^{-3} *M*)	0.43	...	8.0
PGA, alanine	0.10	...	2.0
PGA, DPN, alanine, ATP (20 μM)	0.67	...	13.2
3-P-Pyr (3 μM)‡	1.08	...	36.0
3-P-Pyr, alanine (7.5 μM)	1.23	...	41.0

* PGA = 3-phosphoglyceric acid.
† 0.2 *M* Tris buffer (pH 7.4).
‡ 3-P-Pyr = 3-phosphohydroxypyruvic acid.

TABLE 5

SERINE FORMATION FROM DL-GLYCERIC ACID-3-C^{14}*

Additions	Serine (c.p.m.)	Recovery (Per Cent)
None	2,865	0.5
ATP (20 μM)	21,642	4.0
DPN (400 μgm.)	4,858	0.8
Alanine (20 μM)	5,865	1.1
Alanine, DPN	8,129	1.6
Alanine, DPN, ATP	60,750	12.1

* Incubations with 5×10^5 c.p.m. of DL-glyceric acid-3-C^{14}.

Serine Formation from 3-Phosphohydroxypyruvic Acid.—When 3-phosphohydroxypyruvic acid was incubated with the enzyme, serine synthesis was observed, and this was accelerated by the addition of alanine (Table 4).

DISCUSSION

It is a well-known fact that serine and glycine are readily interconvertible,[10–12] but, from the viewpoint of actual serine synthesis in animal tissues, it appears that certain three-carbon compounds, derived from glycolysis, are more likely to be major precursors of serine.[13] Anker suggested the intermediary formation of serine from pyruvate on the pathway to glycine,[9] but our results do not support this suggestion. Sallach presented a possible scheme which shows serine formation from D-glyceric acid through 3-hydroxypyruvic acid.[4] As mentioned above, D-glyceric acid itself was a poor source and required DPN, alanine, and especially ATP to produce good results. These findings suggest that D-glyceric acid may be

converted to serine through 3-phosphoglyceric acid. As shown in Tables 3 and 4, 3-phosphoglyceric and 3-phosphohydroxypyruvic acid are converted to serine by the additions of DPN and alanine, and alanine, respectively. Although ATP showed a slight activation on the formation of serine from 3-phosphoglyceric acid, it is likely that this is due to the protection of 3-phosphoglyceric acid from dephosphorylation.

On the basis of the above-cited results we present the following alternative pathway of serine biosynthesis (Fig. 1). This pathway might involve the following

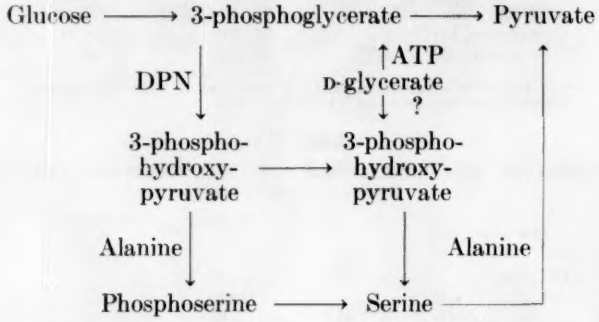

FIG. 1.—Possible pathway of serine formation.

enzymes: a glyceric acid kinase, a 3-phosphoglyceric acid dehydrogenase, a 3-phospho- or hydroxypyruvic acid transaminase, and a phosphatase. From Figure 1 it is seen that one point which still remains unsettled is the identity of the precursor of serine, 3-hydroxypyruvic acid, or phosphoserine. We hope to determine this in the near future.

SUMMARY

1. It was found that a crude enzyme system of rat liver can catalyze serine formation from glucose, D-glyceric, 3-phosphoglyceric, and 3-phosphohydroxypyruvic acids.

2. It was necessary to add ATP, DPN, and alanine to secure synthesis from D-glyceric acid; DPN and glutamate or alanine from 3-phosphoglyceric acid; and alanine from 3-phosphohydroxypyruvic acid.

3. A possible pathway of serine biosynthesis is presented and discussed.

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† On leave from the Institute of Microbial Diseases, Osaka University, Osaka, Japan.

[1] R. J. Wintzler, K. Moldave, M. F. Rafelson, Jr., and H. E. Peatson, *J. Biol. Chem.*, **199**, 485, 1952.

[2] R. Steel, *J. Biol. Chem.*, **198**, 237, 1952.

[3] A. Fischer, G. Fischer, C. Landschütz, G. Ehrensvärd, M. E. Rafelson, Jr., and R. Stjernholm, *Acta Physiol. Scand.*, **27**, 247, 1953.

[4] H. J. Sallach, in W. B. McElroy and B. Glass (eds.), *A Symposium on Amino Acid Metabolism* (Baltimore: Johns Hopkins Press, 1955), p. 782.

[5] The synthesis of this compound by Dr. C. E. Ballou, of the Department of Biochemistry on this campus, has led to a crystalline derivative that has not yet been obtained in an analytically defined form. Its identity, at present, is concluded from the mode of synthesis and from the general properties of the product.

[6] P. Siekevitz and D. M. Greenberg, *J. Biol. Chem.*, **180**, 845, 1949.
[7] W. R. Frisell, L. A. Meech, and C. G. Mackenzie, *J. Biol. Chem.*, **207**, 709, 1954.
[8] O. H. Lowry and J. A. Lopez, *J. Biol. Chem.*, **162**, 421, 1946.
[9] H. S. Anker, *J. Biol. Chem.*, **176**, 1337, 1948.
[10] D. Shemin, *J. Biol. Chem.*, **162**, 297, 1946.
[11] T. Winnick, I. Moring-Claesson, and D. M. Greenberg, *J. Biol. Chem.*, **175**, 127, 1948.
[12] W. Sakami, *J. Biol. Chem.*, **176**, 995, 1948.
[13] H. R. V. Arnstein, *Advances in Protein Chemistry*, **9**, 1, 1954.

DERIVATIVES OF MENADIONE AS ELECTRON ACCEPTORS IN THE OXIDATION OF REDUCED DIPHOSPHOPYRIDINE NUCLEOTIDE

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The results of recent reports suggest a relationship between naphthoquinones and electron transport. Martius and collaborators[1-3] observed a stimulation of electron transport and oxidative phosphorylation by vitamin K_1 and an uncoupling of oxidative phosphorylation by 3,3′-methylenebis-(4-hydroxycoumarin) in studies of animal tissue preparations. Wosilait and Nason,[4] Dolin,[5] and Cormier and Totter[6] reported an acceleration of the rate of oxidation of reduced diphosphopyridine nucleotide (DPNH) by 2-methyl-1,4-naphthoquinone (menadione) in protein preparations obtained from microörganisms. According to a report by Uehara and Muramatsu,[7] the oxidation of phosphogluconate, glucophosphate, and ribose phosphate by yeast autolysates is promoted by menadione. Ball *et al.*[8] observed an inhibition of the reduction of cytochrome c by 2-alkyl-3-hydroxynaphthoquinones and suggested that the inhibition occurred between cytochromes b and c. The inhibition of the reduction of cytochrome c by 2,3-dimercaptopropanol (BAL) observed by Slater[9] and by antimycin A shown by Chance,[10] and the uncoupling of oxidative phosphorylation by 3,3′-methylene bis-(4-hydroxycoumarin),[1] are suggestive of inhibition of a factor or factors which function in electron transport. The inhibitors BAL,[9] antimycin A,[10] and the 2-alkyl-3-hydroxynaphthoquinones[8] appear to exert their effects at the same site.

Chance[11] found that the cytochrome b content of Ehrlich, Krebs 2, and dba thymoma ascites tumors was markedly low as determined by spectroscopic measurement. Strength and Seibert[12] reported that the addition of cytochrome b and menadione to preparations of lymphosarcoma 6C3HED markedly increased the rate of cytochrome c reduction by DPNH, while cytochrome b or menadione alone was ineffective. Dicoumarol inhibited cytochrome c reduction under these conditions and the inhibition was reversed by further additions of menadione. The site of stimulation of the reduction of cytochrome c by menadione and the inhibition by dicoumarol was localized between cytochromes b and c. Reif *et al.*[13] and Reif and Potter[14] in studies of certain normal and tumor tissues obtained results suggestive of antimycin-sensitive and antimycin-insensitive pathways of DPNH oxidation. All the tissues studied exhibited DPNH oxidation

insensitive to antimycin A, while tumor tissues exhibited a very low amount of antimycin-sensitive oxidation. A flavoprotein which oxidizes DPNH, DPN cytochrome reductase, was found to be concentrated in the microsomal fraction of rat liver by Hogeboom,[15] Brody *et al.*,[16] and Strittmatter and Ball.[17] This flavoprotein, obtained in a high degree of purity from pigeon breast muscle by Edelhoch *et al.*[18] and from pig heart muscle by Vernon *et al.*[19] was not inhibited by antimycin A. DPHH oxidase described by Green *et al.*[20] and Mackler[21] was found in mitochondria and was inhibited by antimycin A.[21] This DPNH oxidase is spectroscopically similar to the cytochrome b preparation of Eichel *et al.*,[22] which Strittmatter and Ball[17] found in mitochondria. Since the reduction of cytochrome c is inhibited by BAL, 2-alkyl-3-hydroxynaphthoquinone, dicoumarol, and antimycin A and the rate of reduction of cytochrome c is increased by vitamin K_1[3] or menadione, it does not appear unlikely that these compounds are exerting their effects upon the same component of electron transport. Furthermore, the reports suggest a major pathway of DPNH oxidation in mitochondria of animal tissues which is capable of transferring electrons to cytochrome c.

Since some of these reports show an effect of naphthoquinones on electron transport, the preparation of derivatives of menadione and their influence on an electron transport system are reported.

Methods.—Powders of C3H mouse liver and lymphosarcoma 6C3HED were prepared from freshly excised tissue by homogenizing the liver or tumor in 20 volumes of acetone for 60 seconds in a Waring Blendor at −15° C. The precipitated tissue was collected on a filter paper in a Buchner funnel, washed twice with cold acetone, and dried in a vacuum desiccator. The dried preparations were powdered in a mortar and stored *in vacuo* at 4° C. Although the activities of the powders were only 50 per cent of those of fresh tissues, the activities were fully maintained for several weeks. Tissue extracts were prepared immediately before use by homogenizing 200 mg. of tissue powder in 5 ml. of distilled water in a Potter-Elvehjem homogenizer and centrifuging the homogenate at 13,500 × g for 10 minutes. The supernatant fraction was used as the enzyme source, and the process of centrifugation obviated the necessity of adding cyanide, which Keilin and Hartree[23] have shown inhibits cytochrome oxidase, thus permitting a direct measure of the reduction of cytochrome c without the addition of cyanide.

In preliminary experiments, the addition of menadione or other naphthoquinone derivatives to cytochrome c resulted in no changes in the absorption spectrum of cytochrome c in the vicinity of 550 mμ.

Cytochrome b† was prepared according to the method of Eichel *et al.*,[22] and lactic acid dehydrogenase was prepared by the procedure of Sumner and Krishnan.[24] Protein concentrations were determined by the method of Lowry *et al.*[25]

S-(2-methyl-1,4-naphthoquinoyl-3) glutathione was prepared according to Fieser and Fieser;[26] S-(2-methyl-1,4-naphthoquinoyl-3) thioglycolic acid and S-(2-methyl-1,4-naphthoquinoyl-3) homocysteine were prepared according to Fieser and Turner;[27] 2-methyl-3-hydroxy-1,4-naphthoquinone and 2-methyl-1,4-naphthoquinone-2,3-oxide were prepared according to Fieser;[28] the 4-monoxime of 2-methyl-1,4-naphthoquinone was prepared by the method of Anderson and Newman;[29] 2,3-dimethyl-1,4-naphthoquinone, β-(2-methyl-1,4-naphthoquinoyl-3) propionic acid, 2-methyl-3-phytyl-1,4-naphthoquinone, and 2-methyl-3-phytyl-1,4-naphthohydro-

quinone diacetate were products available in this laboratory. The purity of these latter products was established by their characteristic melting points. 2-Methyl-1,4-naphthoquinone sodium bisulfite was a commercial product ("Hykinone," Abbott Laboratories).

Results.—Extracts prepared from tissue powders by homogenization and centrifugation for 10 minutes at 13,500 × g reduced cytochrome c. The comparative data presented in Table 1 show that liver extract without added cytochrome b is three times as active as tumor extract, and liver extract without added menadione is seven times as active as tumor extract. However, tumor extract was nearly as active as liver extract when all reactants were added. Maximum activity was possible only when DPN, cytochrome b, menadione, and substrate were added. Data are included to demonstrate that without cytochrome c a maximum activity response was obtained at about 0.3 μM of added menadione.

TABLE 1

REDUCTION OF CYTOCHROME C IN LACTIC ACID DEHYDROGENASE SYSTEM; REQUIREMENT FOR CYTOCHROME B, MENADIONE, AND DPN

	Additions to System*				
Tissue	Na Lactate (μM)	DPN (μM)	Cytochrome b (μg. Protein)	Menadione (μM)	Activity Units†
Liver	445	0.2	0	0	67
	445	0.2	0	0.3	134
	445	0.2	110	0	74
	445	0.2	110	0.3	382
Tumor	0	0.2	110	0.3	15
	445	0.2	0	0	8
	445	0	110	0.3	10
	445	0.2	0	0.3	41
	445	0.2	110	0	10
	445	0.2	110	0.3	270
	445	0.2	0	0.15	26
	445	0.2	0	0.30	42
	445	0.2	0	0.45	44

* Other components of the system were 1.0 ml. of 0.5 *M* phosphate buffer, pH 7.4; 0.4 ml. of 1×10^{-4} *M* cytochrome c. Total volume, 3.0 ml.

† One unit = $\Delta \log I_0/I$ of 0.001 during the minute between 30 and 90 seconds, measured at 550 mμ for 1.0 mg. tissue protein.

Since these studies established the conditions under which menadione accelerated the reduction of cytochrome c, it was of interest to determine whether dicoumarol or BAL inhibited the response to menadione. The presence of 0.6 μM of dicoumarol in the system containing 0.3 μM of menadione inhibited the rate of reduction of cytochrome c about 50 per cent, and 0.6 μM of dicoumarol in the presence of 1.0 μM of menadione was noninhibitory. In our experiments BAL reduced cytochrome c directly, thus making it difficult to determine any direct effect on the enzyme systems. However, BAL reacted readily with menadione in alcohol at room temperature to give a brown product. Since the reaction product was not obtained as a crystalline substance, it was purified as a brown oil, and this product was found to be inactive in the enzyme system.

The results presented in Table 2 illustrate the comparative effectiveness of derivatives of menadione in accelerating the rate of cytochrome c reduction by tumor extract. The data reported were obtained without the addition of cytochrome b since the extracts show a marked response to menadione or derivatives without the addition of cytochrome b. The addition of menadione and derivatives of mena-

dione, such as the derivatives containing glutathione, thioglycolic acid, and L-homocysteine with the thioether linkage at the 3-position, resulted in a two- to threefold increase in the rate of reduction of cytochrome c. The DL-homocysteine derivative was approximately 50 per cent as effective as the other active derivatives. The L-homocysteine derivative was about three times as active as the D-homocysteine derivative. Derivatives with substituents joined to the 3-position through linkages other than the thioether linkage were inactive.

Although the response to menadione and the naphthoquinone derivatives with thioether linkages is several fold greater in the presence of added cytochrome b complete data are not available on all the compounds. However, 2-methyl-1,4-naphthoquinone, the 3-glutathione, the 3-thioglycolic acid, the 4-monoxime, and the 3-hydroxy derivatives of menadione have been studied in a system containing added cytochrome b. The results of these studies suggested the same pattern of activity and inactivity of the derivatives as is shown in Table 2.

TABLE 2

COMPARISON OF EFFECTIVENESS OF DERIVATIVES OF 2-METHYL-1,4-NAPHTHOQUINONE IN REDUCTION OF CYTOCHROME C IN LACTIC ACID DEHYDROGENASE SYSTEM

Compound Added*	Activity Units†
None	10
2-Me-1,4-NQ	46
S-(2-Me-1,4-NQ-3) glutathione	41
S-(2-Me-1,4-NQ-3) thioglycolic acid	39
S-(2-Me-1,4-NQ-3) DL-homocysteine	26
S-(2-Me-1,4-NQ-3) D-homocysteine	18
S-(2-Me-1,4-NQ-3) L-homocysteine	32
2-Me-3-OH-1,4-NQ	13
2-Me-1,4-NQ-2,3-oxide	8
2,3-diMe-1,4-NQ	8
2-Me-1,4-NQ-4-monoxime	8
β-(2-Me-1,4-NQ-3) propionic acid	10
2-Me-3-phytyl-1,4-NQ	10
2-Me-3-phytyl-1,4-NHQ diacetate	8
Hykinone	8

* 0.3 μM of each derivative was added to the system containing 1.0 ml. of 0.5 *M* phosphate buffer, pH 7.4; 0.4 ml. of 1×10^{-4} *M* cytochrome c; 445 μM of sodium lactate; 0.2 μM of DPN. Me = methyl; NQ = naphthoquinone; NHQ = naphthohydroquinone; OH = hydroxyl.

† One unit = $\Delta \log I_0/I$ of 0.001 during the minute between 30 and 90 seconds, measured at 550 mμ for 1 mg. of tissue protein.

The data presented in Table 2, which show acceleration of the reduction of cytochrome c by the menadione derivatives containing thioether linkages at the 3-position, the inactivity of menadione derivatives with other substituents at the 3-position, and a specificity for the L-homocysteine derivative, suggested a replacement of the thiol substituent by a sulfhydryl of protein. That chemical replacement of glutathione by thioglycolic acid is possible experimentally was ascertained as follows: 100 mg. of the glutathione derivative (m.p. dec. 173° C.) was dissolved in 25 ml. of water, and to this was added a large excess of thioglycolic acid (2 gm.). After 30 minutes the mixture was diluted to 50 ml. and set in the refrigerator at 0° C.; crystallization began within 10 minutes. After four hours in the refrigerator the crystals were collected on a filter paper and washed repeatedly with distilled water at room temperature. The yield of the dried crystalline product was 34 mg.

(theoretical yield 55 mg.) representing a yield of 62 per cent (m.p. 156°–157°). Fifteen milligrams of the product recrystallized from ethanol and water melted at 157.5°–159°; twice-recrystallized authentic S-(2-methyl-1,4-naphthoquinoyl-3) thioglycolic acid melted at 157°–159°; a mixed melting point of the recrystallized product and twice-recrystallized authentic sample was 157.5°–158.5°; Fieser and Turner[27] reported m.p. 158°–160°, 156°–158°. Solutions of the authentic thioglycolic acid derivative and the isolated reaction product in 95 per cent alcohol (2 mg. per 100 ml.) were subjected to spectral analysis between 220 and 700 mμ. The solutions exhibited absorption maxima at 261 and 422 mμ and minima at 227 and 381 mμ. There was close agreement in the intensity of the absorption of the two solutions at all points.

Since the 4-monoxime of 2-methyl-1,4-naphthoquinone with an unsubstituted 3-position was inactive in the enzyme system, it was desirable to ascertain whether it reacts with sulfhydryl compounds to give a 3-thioether. Incubation of the monoxime with thioglycolic acid in alcohol did not give an addition product, and the unchanged monoxime was recovered in 85 per cent yield by precipitation of the compound with an excess of added water.

In further enzymatic studies, lactic acid dehydrogenase from beef heart was used to replace tissue extract, and the requirements for the reduction of cytochrome c were as follows: sodium lactate, lactic acid dehydrogenase, DPN, cytochrome b, menadione, and cytochrome c. The omission of any single component resulted in little or no reduction of cytochrome c. Lactic acid, lactic acid dehydrogenase, and DPN were replaceable by DPNH, and menadione and cytochrome c were replaceable by the dye 2,6-dichlorophenol indophenol. These observations established that the cytochrome b preparation was capable of oxidizing DPNH in the presence of suitable electron acceptors, such as menadione or 2,6-dichlorophenol indophenol. The results presented in Table 3 show the effect of menadione or the dye on the

TABLE 3

EFFECT OF MENADIONE OR 2,6-DICHLOROPHENOL INDOPHENOL ON THE OXIDATION OF DPNH BY PIG HEART CYTOCHROME B

Additions to System*			Activity†	
Cytochrome b Protein (μg.)	Menadione (mμM)	Dye (mμM)	Dye Units	DPN Units
110	0	0		3.0
0	200	0		0.5
110	200	0		19.3
0	0	200	0.2	
28	0	200	4.7	
55	0	200	9.9	
110	0	200	18.8	
165	0	200	28.9	

* The reactions were carried out in a reaction mixture containing the above components; 1.0 ml. of 0.5 *M* phosphate buffer, pH 7.4; and 250 millimicromoles of DPNH prepared by a modification of the method of M. E. Pullman, S. P. Colowick, and N. O. Kaplan, *J. Biol. Chem.*, **194**, 593, 1952. Total volume, 3.0 ml.

† One DPN unit = millimicromoles of DPNH oxidized per minute per 3 ml. = $E_{340} \times 161 \times 3$. One dye unit = millimicromoles of dye reduced per minute per 3 ml. = $E_{600} \times 62.5 \times 3$ measured in a Beckman DU spectrophotometer in a total volume of 3 ml. in quartz cuvettes with a light path of 1.00 cm.

oxidation of DPNH by cytochrome b. The addition of several levels of cytochrome b demonstrated the linear response to enzyme. The enzyme showed very little activity in the absence of electron acceptors, but the addition of 200 millimicromoles of menadione and indophenol gave activities of 19.3 and 18.8 units, respec-

tively. Thus the two compounds are equivalent as acceptors of electrons under the conditions described. Very little oxidation of reduced DPN was observed in the absence of enzyme.

Discussion.—Lymphosarcoma 6C3HED exhibited a low capacity for the reduction of cytochrome c in the oxidation of lactic acid. Added cytochrome b and menadione increased the rate of reduction of cytochrome c.

Pig heart cytochrome b[22] exhibiting absorption maxima at 414 mμ in the oxidized state and at 428, 525, and 560 mμ in the reduced state possessed DPNH oxidase activity and, in the presence of menadione, catalyzed the reduction of cytochrome c. This pig heart cytochrome b is spectroscopically similar to the DPNH oxidase from beef heart mitochondria described by Mackler.[21] Chance[10] described experimental conditions required for the oxidation of DPNH by cytochrome b, using starved yeast cells as the source of enzyme. Antimycin A inhibited DPNH oxidation[21] and cytochrome c reduction.[10] BAL inhibited cytochrome c reduction,[9] and the results of our experiments suggest that BAL inhibits the reduction of cytochrome c by combining with 2-methyl-1,4-naphthoquinone or a physiological quinone of similar chemical properties.

A study of the activity of derivatives of menadione gave results which indicated that the 3-position of 2-methyl-1,4-naphthoquinone is of primary importance. Menadione and the thioether derivatives of L-homocysteine, thioglycolic acid, and glutathione were active. Activation of the system by thioether compounds may be dependent upon enzymatic cleavage of the thioether or a replacement reaction in the presence of sulfhydryl compounds. Enzymatic cleavage may be suggested by our observation (Table 2) showing specificity for the L-homocysteine derivative. A replacement reaction wherein the sulfhydryl of protein replaces the thiol substituent on the 3-position of the naphthoquinone may be suggested by the results demonstrating chemical replacement of glutathione of S-(2-methyl-1,4-naphthoquinoyl-3) glutathione by thioglycolic acid. The 4-monoxime of menadione was inactive in the enzyme system and did not react with thioglycolic acid. Other derivatives of menadione with the 3-position substituted with alkyl, acyl, hydroxyl, and oxide groups which were not replaceable by sulfhydryl groups were inactive. The inhibition of DPNH oxidation by dicoumarol and reversal by menadione, previously reported,[4] has been confirmed.

The data reported suggest a pathway of DPNH oxidation catalyzed by a mitochondrial enzyme spectroscopically similar to cytochrome b[22] which is sensitive to antimycin A[21] and BAL and which will oxidize DPNH and will reduce cytochrome c in a system containing certain quinoid structures.

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† Several cytochrome b preparations were supplied through the courtesy of Dr. S. J. Cooperstein, Western Reserve University, Cleveland, Ohio.

[1] C. Martius and D. Nitz-Litzow, *Biochem. et biophys. acta*, **12**, 134, 1953.

[2] C. Martius and R. Strufe, *Biochem. Z.*, **326**, 24, 1954.

[3] C. Martius, *Biochem. Z.*, **326**, 26, 1954.

[4] W. D. Wosilait and A. Nason, *J. Biol. Chem.*, **208**, 785, 1954.

[5] M. I. Dolin, *Biochem. et biophys. acta*, **15**, 153, 1954.

[6] M. J. Cormier and J. R. Totter, *J. Am. Chem. Soc.*, **76**, 4744, 1954.

[7] K. Uehara and I. Muramatsu, *Proc. Japan Acad.*, **29**, 511, 1953; cited from *Chem. Abstr.*, **49**, 1112[e], 1955.

[8] E. G. Ball, C. B. Anfinsen, and O. Cooper, *J. Biol. Chem.*, **168**, 257, 1947.
[9] E. C. Slater, *Biochem. J.*, **45**, 14, 1949.
[10] B. Chance, in W. D. McElroy and D. Glass (eds.), *The Mechanism of Enzyme Action* (Baltimore: Johns Hopkins Press, 1954), p. 399.
[11] B. Chance, *Science*, **116**, 200, 1952.
[12] D. R. Strength and M. A. Seibert, *Proc. Am. Assoc. Cancer Research*, **2**, 49, 1955.
[13] A. E. Reif, V. R. Potter, and G. A. LePage, *Cancer Research*, **13**, 807, 1953.
[14] A. E. Reif and V. R. Potter, *Arch. Biochem. and Biophys.*, **48**, 1, 1954.
[15] G. H. Hogeboom, *J. Biol. Chem.*, **177**, 847, 1949.
[16] T. M. Brody, R. I. H. Wang, and J. A. Bain, *J. Biol. Chem.*, **198**, 821, 1952.
[17] C. F. Strittmatter and E. G. Ball, *J. Cellular Comp. Physiol.*, **43**, 57, 1954.
[18] E. Edelhoch, O. Hayaishi, and L. J. Tepley, *J. Biol. Chem.*, **197**, 97, 1952.
[19] L. P. Vernon, H. R. Mahler, and N. K. Sarkar, *J. Biol. Chem.*, **199**, 599, 1952.
[20] D. E. Green, B. Mackler, R. Repaske, and H. R. Mahler, *Biochem. et biophys. acta*, **15**, 435, 1954.
[21] B. Mackler, *Federation Proc.*, **11**, 248, 1955.
[22] B. Eichel, W. W. Wainio, P. Person, and S. J. Cooperstein, *J. Biol. Chem.*, **183**, 89, 1950.
[23] D. Keilin and E. F. Hartree, *Proc. Roy. Soc. London, B*, **125**, 171, 1938.
[24] J. B. Sumner and P. S. Krishnan, *Enzymologia*, **12**, 232, 1948.
[25] O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265, 1952.
[26] L. F. Fieser and M. Fieser, *Organic Chemistry* (Boston: D. C. Heath & Co., 1944), p. 728.
[27] L. F. Fieser and R. B. Turner, *J. Am. Chem. Soc.*, **69**, 2335, 1947.
[28] L. F. Fieser, *J. Biol. Chem.*, **133**, 391, 1940.
[29] R. J. Anderson and M. S. Newman, *J. Biol. Chem.*, **103**, 405, 1933.

METABOLIC PECULIARITIES IN NORMAL YOUNG MEN AS REVEALED BY REPEATED BLOOD ANALYSES

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Physiologists and biochemists have for decades been engaged in trying to construct a *single picture* which will represent the way in which the body of a normal human being functions. As a result of this approach, a tremendous volume of information and insight has been amassed, which, of course, it would be impossible to summarize in a short space. The task of constructing this picture, even leaving out all complications, is an extremely difficult one and very far from completed. It needs to be carried forward continuously.

This approach, however, leaves out of consideration certain serious complications to which we wish to draw attention. Largely because of our ardent attempts to develop a statistically valid *single picture*, a tremendous number of physiological difficulties which human beings encounter are almost as little understood today as they were decades ago, before we became acquainted with microbial infections, virus infections, and specific nutritional deficiencies. We have learned to classify the many different kinds of invading organisms and viruses and to recognize among them not only species differences but important and crucial strain differences. We have made very little progress, however, in learning how to classify people with respect to their susceptibility to metabolic or infective diseases; yet we recognize

in an academic way that variation within the human family is very great and that, regardless of whether the disease is metabolic or infective, marked differences in susceptibility exist.

Our reference to the little-understood physiological difficulties which people often encounter has to do with medical problems: multiple sclerosis, muscular dystrophy allergies, arthritis, arteriosclerosis, heart failure, cancer, and various forms of mental disease, including schizophrenia. While for many of these diseases we may know the immediate precipitating causes and may be able to institute palliative measures we are still very much in the dark as to the fundamental bases on which the existence of these diseases rests.

This darkness may gradually be dispelled as the result of innumerable research contributions which are not consciously or specifically related to the central theme of our discussion, but the desired end will come much more rapidly if we physiologists and biochemists can be diverted sufficiently from our search for the attributes of a normally functioning individual so that some substantial attention can be devoted to studying the distinctive attributes possessed by real individuals.

We submit as a working hypothesis the idea that there is only one place to search for the key attributes which make certain individuals susceptible to arthritis, allergies, heart disease, cancer, mental disease, etc.; this is *in individuals*. By concentrating on the statistical "single picture" referred to above, we bar ourselves from finding the distinctive attributes which make for susceptibility to numerous specific diseases, including many that laymen rarely hear of.

In this connection it is well to remind ourselves that susceptibility to disease concerns the majority; it is not a minority problem. Most individuals exhibit susceptibility to one or more diseases, and this susceptibility is often of a specific nature. Obstetricians are happy when they can inform the mother that her baby is "normal." But when normal infants mature, they usually develop into adults who, because of specific susceptibilities, become diseased.

What is needed, in the authors' opinion, is a new approach which will utilize the vast knowledge that has accumulated but which will disregard, for the time being, the time-honored philosophy which centers its attention on the normal (reference) man. When we study chemistry, we learn about the behavior of all kinds of chemicals, their similarities and their differences. When we study microbiology, likewise, we learn about microörganisms, their likenesses and their differences. We carry out our studies of these subjects for the most part inductively—that is, we make observations first, and then we formulate our generalizations on the basis of these observations. When in human physiology we adopt the philosophy of the "single picture," the reference man, we reverse this procedure; we first *assume* that the reference individual is the center of interest; then we make observations and apply statistics in an attempt to find out what the reference individual is like.

The importance of this single-picture philosophy is indicated by the fact that it is implicitly accepted by the authors of practically every treatise and monograph dealing with physiology, biochemistry, pharmacology, and physiological psychology and by the authors of most journal articles in these fields.

The purpose of the present investigation is to make a beginning in a long-range inductive approach which we are confident will contribute greatly to the understanding of diseases of which the etiologies are now obscure. We have studied

inductively and individually a group of young men who are at present well and normal, with the full realization that they may have in them the roots of disease susceptibility, particularly susceptibility to metabolic disease. We have explored within this group of well young men for the existence of distinctive blood characteristics which may be associated with disease susceptibility or disease resistance.

At this stage in the development of this new approach, we regard the mere existence of metabolic peculiarities in so-called "normal" young men as sufficiently important to merit attention. To postulate or guess what diseases specific peculiarities may foreshadow would be abandoning induction for premature deduction. If we can offer convincing evidence that distinctive and suggestive attributes exist in young men who are at present healthy, other investigators will be induced to follow this lead by searching in a hundred ways for these individual characteristics which are probably closely related to disease susceptibility. Research directed to this end has in the past been practically nonexistent. While much research in the broad area which includes physiology, biochemistry, and physiological psychology has necessarily been concerned with individuals, the information obtained has been regarded as valuable and scientific only when it applies to the reference man.

A total of twelve healthy young men were involved in this study.[1] Of these, eight were studied more thoroughly than the other four. From each of the eight individuals, five or six fasting samples of blood were drawn at weekly or longer intervals, and each sample was analyzed by conventional, recognized clinical methods for glucose, lactic acid, creatinine, urea, uric acid, amylase, lipase, acid phosphatase, alkaline phosphatase, acetyl cholinesterase, inorganic phosphate, sodium, potassium, calcium, and magnesium. The corpuscles from each blood sample were also analyzed for sodium, potassium, calcium, and magnesium. The character of the results can best be judged by a consideration of Figures 1–9.

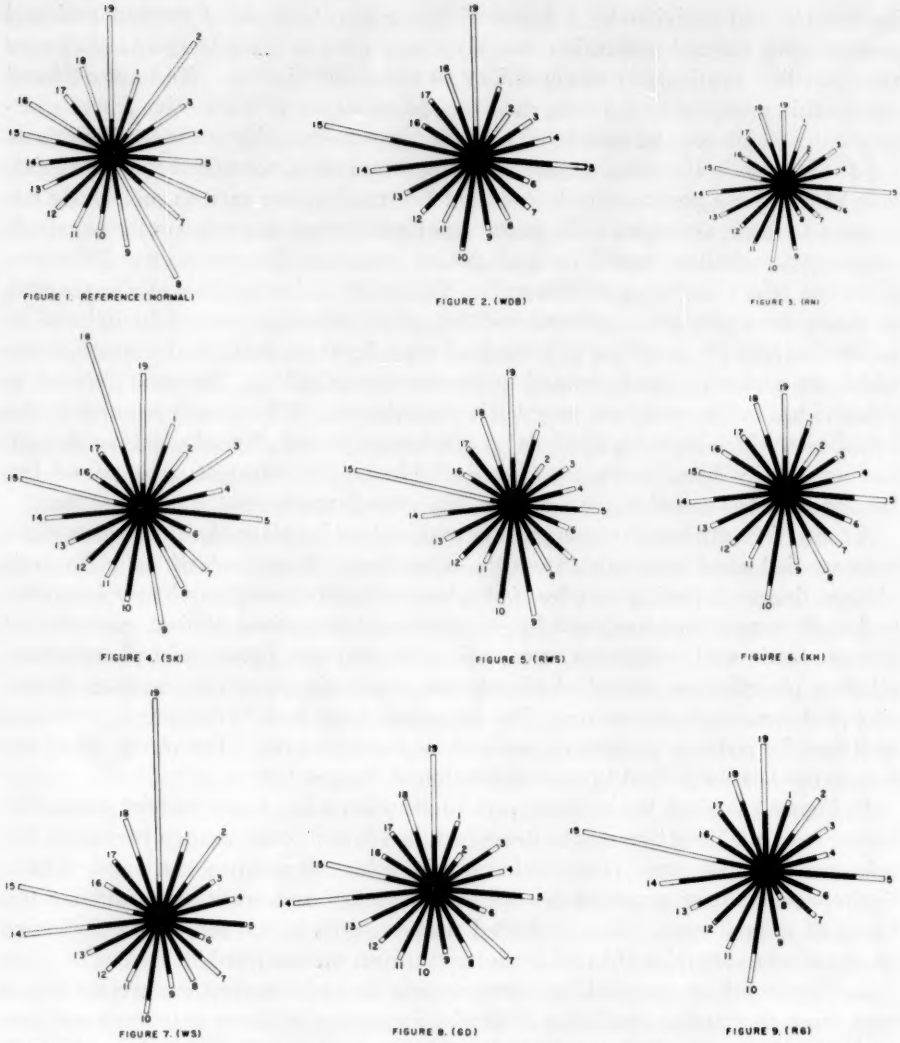
In Figure 1 is given the accumulated information taken from the best obtainable values from the literature on the items included in our study as they pertain to the reference (normal) man. Graphed in terms of polar co-ordinates, the length of each line represents on an appropriate scale the amount of each constituent found in the blood of normal man. The unshaded portion of each line represents the range observed when samples of blood from normal men are analyzed.

In Figures 2–9 are graphed the corresponding data with respect to the eight young men most thoroughly studied. A careful inspection of these graphs shows that each one of the eight individuals exhibits at least one peculiarity of the sort which may be important in connection with his distinctive susceptibility or resistance to a specific metabolic (or infective) disease. The specific peculiarities which stand out in this study are listed in Table 1.

TABLE 1

OBSERVED PECULIARITIES IN BLOOD COMPOSITION

High	Low	Highly Variable	Conspicuously Constant
Plasma Mg (WDB)	Plasma Mg (KH)	Plasma Mg (RWS)	Cell Na (WDB)
Plasma K (RN)		Plasma Ca (WS)	Cell Ca (RG)
Uric acid (SK)		Cell Ca (SK)	
Alkaline phosphatase (RWS)		Creatinine (SK)	
		Lipase (GD)	
		Acetylcholinesterase (RN)	



FIGS. 1–9.—Blood components: *1*, glucose; *2*, lactic acid; *3*, creatinine; *4*, urea; *5*, uric acid; *6*, amylase; *7*, lipase; *8*, acid phosphatase; *9*, alkaline phosphatase; *10*, acetylcholinesterase; *11*, inorganic phosphate; *12*, sodium (plasma); *13*, potassium (plasma); *14*, calcium (plasma); *15*, magnesium (plasma); *16*, sodium (cell); *17*, potassium (cell); *18*, calcium (cell); *19*, magnesium (cell).

The inclusion of "highly variable" items and "conspicuously constant" items among the observed peculiarities is justified, first, by the fact that they unquestionably stand out as distinctive for the individuals concerned and, second, by the fact that there are already strong hints, from work both in our laboratory[2] and elsewhere,[3] that this type of peculiarity may be significant in connection wth disease susceptibility.

Elementary statistical considerations make our findings reasonable. The idea that a normal young man will exhibit about average values when tested in a number

of different independent ways is bound to be in error. An individual young man selected at random will have one chance in two of being within the median 50 per cent with respect to his fasting blood glucose level. He will have one chance in four of being within the median 50 per cent with respect to his glucose level *and* his lactic acid level. He will have one chance in eight of being within the median 50 per cent with respect to three independent items. When one considers as many as nineteen items, the number we have included in our study, the chance of any individual's being within the middle 50 per cent range with respect to all nineteen items (assuming that they are independent measures) would be less than one in a half-million.

These considerations bring into bold relief the important difference between "statistical man" and any individual man. Statistical man, by definition, is about average in every respect; real men are likely to deviate markedly from the average in many respects. If it were possible to produce a synthetic statistical man, there is no telling what he would be like. Undoubtedly he would be extraordinary; possibly he would lack susceptibility to disease. Real individuals, on the other hand, have peculiarities in metabolism as well as disease susceptibilities, and it seems reasonable to suppose, as a working hypothesis, that the peculiarities and the susceptibilities are closely related. It also seems reasonable to hope, on the basis of the genetotrophic concept,[4] that many diseases of obscure etiology can be successfully attacked once we are acquainted with their biochemical nature.

The fact that enzyme levels in the blood are characteristically different for different individuals clearly indicates that the body chemistry of each individual is distinctive. While the total metabolism of each of two men of about average height and weight, measured in calories, may be about the same and very close to that of statistical man, the details of the metabolism of each may be highly distinctive. Some specific chemical reactions may be taking place in one individual ten times as fast as they are in another.[5] If this is true, surely this must be the basis for differences in disease susceptibility.

It is perhaps belaboring the obvious to say that there are a multitude of ways in which these or any other young men could be studied, quite aside from our particular study. Each method of study would reveal additional evidence of the importance of individuality—a concept which commonly receives lip service but very little scientific attention.[6]

If the concept of statistical man is of limited significance in connection with susceptibility to metabolic diseases, it is also of limited value in many other areas in which human problems arise. It is no wonder that, in the social sciences generally, the concept of humanity is based upon the scientifically determined characteristics of statistical man as postulated by the biological sciences. There must be a revolution in our thinking not only in medicine but in the social field as well; statistical man must be dethroned from his position as the center of our interest. On the basis of our study and observations he finds no counterpart in a population of real individuals. If we think that a typical human population consists of individuals who are "about average" in most respects, our thinking is bound to be unrealistic and futile, because a typical human population contains no such individuals.

[1] R. W. Shideler, Ph.D. thesis, University of Texas, Austin, 1955; W. D. Brown, Ph.D. thesis, University of Texas, Austin, 1955.

[2] M. K. Young, H. K. Berry, E. Beerstecher, Jr., and J. S. Berry, *University of Texas Publication No. 5109* ("Biochemial Institute Studies," Vol. 4 [Austin: University of Texas, 1951]), p .189.

[3] L. Bellak, *Dementia Praecox* (New York: Grune & Stratton, 1948), p. 27.

[4] R. J. Williams, E. Beerstecher, Jr., and L. J. Berry, *Lancet*, p. 287, February 18, 1950.

[5] R. J. Williams, *Origins of Resistance to Toxic Agents* (New York: Academic Press, Inc., 1955), p. 200.

[6] R. J. Williams, *Free and Unequal* (Austin: University of Texas Press, 1953).

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*NYBOMYCIN, A NEW ANTIBIOTIC WITH ANTIPHAGE AND ANTIBACTERIAL PROPERTIES**

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Culture liquids of a streptomycete, A 717, isolated from Missouri soil, were reported earlier to have a broad antiphage spectrum. Concentrates prepared from the culture liquid were found to have no effect on PR8 influenza or on SK polio virus.[1]

In further investigations a colorless crystalline compound with antiphage and antibacterial activity was isolated from the culture liquid and mycelium of A 717. It has been named *nybomycin.* The production, isolation, and some of the properties of nybomycin are described in this report.

Culture and Production.—The streptomycete A 717 was maintained on oatmeal agar. On agar plates the culture was tan to brownish, with slight droplet production; the undersurface was pink to reddish brown. For production of nybomycin the organism was grown in submerged aerated culture at room temperature in a potato extract medium.[2] Antiphage activity generally appeared on the third or fourth day, at which time the pH of the culture fluid was 7.2–8.2. The active substance was present in the mycelium and in the culture liquid in about equal amounts, the proportion varying with the age of the culture at the time of harvest.

Purification and Chemical Properties.—Isolation and purification of nybomycin was followed by assay with *Bacillus cereus* phage as the test organism, using the paper disk method.[1] The mycelium was collected on a Buchner funnel, washed with water, and then extracted several times with boiling 95 per cent ethanol. The combined extracts were taken to dryness *in vacuo* and treated successively with boiling Skellysolve C (a petroleum ether fraction of boiling range 80–100° C.), cold ether, and cold acetone, all of which removed colored impurities. The residue was extracted with boiling 95 per cent ethanol, which, on cooling, deposited grayish-white crystals of crude nybomycin.

The culture liquid, free of mycelium, was adjusted to pH 6.0 and extracted several times with *n*-butanol, or it was acidified to pH 2.5, filtered, and extracted with one-half its volume of chloroform. Considerable loss was experienced with the latter method because, on acidification, a precipitate containing active material was formed from which the compound could not be isolated. The extracts in bu-

tanol or chloroform, after being taken to dryness *in vacuo* and treated in the same way as described for the mycelium, yielded the same crystalline compound (as determined by ultraviolet spectrum) as obtained from the mycelium.

The crude crystalline material was further purified by dissolving in concentrated hydrochloric acid and treating the solution with charcoal (Norit A). On dilution of the resulting light yellow liquid with 5–10 parts of water, colorless crystals formed immediately. They were dissolved in boiling acetic acid, from which they crystallized on cooling or on addition of water, acetone, or other common organic solvents. Depending on the solvent from which it was recrystallized, nybomycin appeared microscopically as small, thin needles and occasionally as rods or rhomboid forms.

Concentrated hydrochloric acid, in which a 5 per cent solution could be obtained, was found to be by far the best solvent. Presumably, the weakly basic compound forms a yellow hydrochloride which, on dilution with water, reverts to the colorless base. Solubility in boiling acetic acid was one-twentieth of that in hydrochloric acid. Pure nybomycin is even less soluble in the lower alcohols, including sec. butanol and isopropanol, all at boiling point, and only very slightly soluble in boiling water, pyridine, acetonitrile, and methylisobutylketone. It is very slightly soluble in cold water, alkali, and all the common organic solvents.

The ultraviolet spectrum of nybomycin in ethanol shows maxima at 266 and 285 mμ. Spectra of nybomycin in ethanol and in ethanol/concentrated hydrochloric acid (1 : 2) are shown in Figure 1. The infrared spectrum is shown in Figure 2. Analytical data[3] agree with the formula $C_8H_7O_2N$. Found: C, 64.21; H, 4.82; O, 21.60; N, 9.47; molecular weight 164 (ebullioscopic in acetic acid). Calculated for $C_8H_7O_2N$: C, 64.42; H, 4.73; O, 21.46; N, 9.39; molecular weight 149.

Nybomycin can be sublimed *in vacuo* at 250° C. without change in its ultraviolet spectrum or loss of biological activity. It darkens at approximately 330° C. without melting. It gives no color with ferric chloride and does not react in the biuret and ninhydrin test.

Antiphage Activity.—Nybomycin was tested against sixty bacteriophages and one actinophage by the paper disk method.[1] Disks were prepared by dipping them in a hot alcohol solution of nybomycin at 0.1 mg/ml. Antiphage activity was found for fifteen of the phages (staphylophages 1, 2, 3, 5, 8, 11, 14, S3K, muscae; *B. cereus* phage; *Escherichia coli* phages T-2, T-5, and T-6r; paratyphoid poona phage 1; and the phage for *Streptomyces griseus*); partial activity was found for eighteen phages (streptophage 3; enterococcus phages 5/7, 1A, 1B, 2B, and 4; *E. coli* phages T-1, T-3, T-7, and FCZ; paratyphoid A phage; and cholera phages B, D, F, G, J, K, and M). No action was observed on streptophages 4, 6, and 7; *B. megatherium* phage; *B. subtilis* phage C.S.C.; *E. coli* phages 2, 4, 5, 6B, 6C, 8, 49B, 50Br, and L-2; paratyphoid B phage 1; paratyphoid poona phage 2; *Salmonella typhi* phage 1; *Shigella dysenteriae* phages S13 and Flexner 2 and 3; *Pseudomonas* phages 1 and 2; cholera phages C, E, H, and R. Stimulation of *E. coli* phages 24B and 53 alpha was noted. These results agree in general with those reported earlier for the antiphage activity of culture liquids of A 717.[1]

Antiphage activity of an alcoholic solution of nybomycin was determined by serial dilution.[1] The minimum inhibitory concentration for staphylophage 14 was 1.7 μg. per milliliter; for enterococcus phage 5/7, 0.6 μg.; for *B. cereus* phage, 0.5 μg.; for *E. coli* phage T-2, 0.6 μg.; and for the actinophage, 0.2 μg.

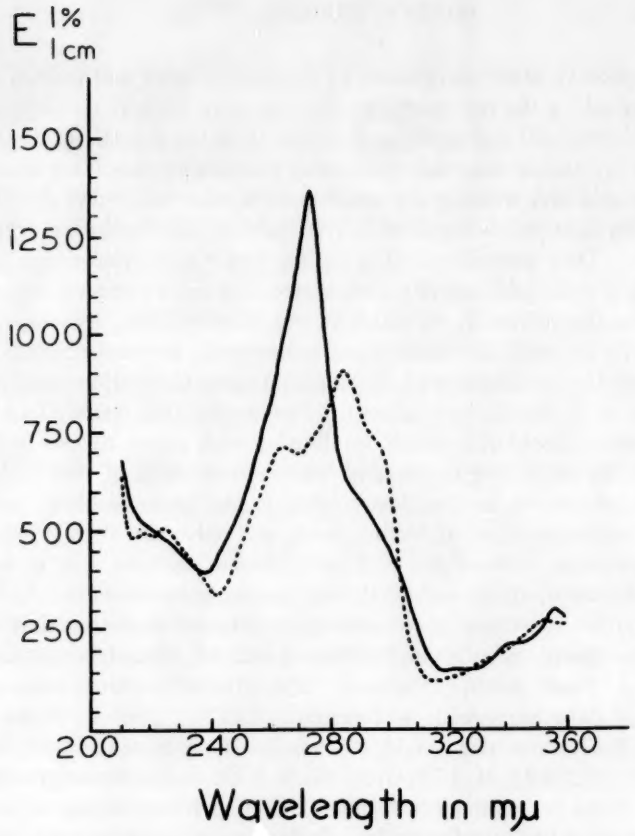

FIG. 1.—Ultraviolet absorption spectrum of nybomycin. Measurements in a Cary recording spectrophotometer, Model 11. *Broken line:* saturated ethanolic solution (approximately 0.025 mg./ml.) diluted with two volumes of ethanol; *solid line:* saturated ethanolic solution diluted with two volumes of concentrated hydrochloric acid.

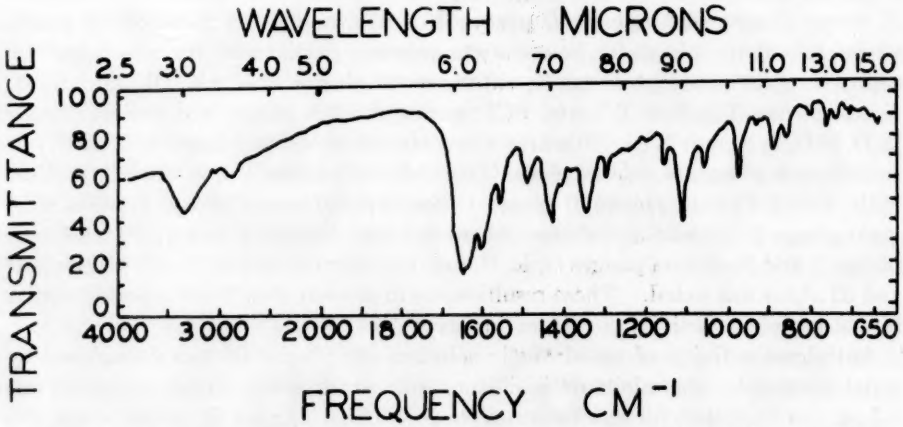

FIG. 2.—Infrared spectrum of nybomycin (KBr briquette) by the Anderson Laboratory, Champaign, Illinois.

The phagocidal effect of nybomycin on free phage particles was determined by the method described by Asheshov *et al.*[1] Because of the relatively slight solubility of nybomycin in suitable solvents, it was suspended in 0.5 per cent sodium carboxymethyl cellulose (CMC). A control tube containing the same concentration of CMC was included in each test, and the total number of phage particles in this tube was considered as 100 per cent.

Nybomycin at 1 mg/ml. had no effect on staphylophage 14, enterococcus phage 5/7, *E. coli* phages T-2 and FCZ, cholera phage D, and the actinophage. Exposure of *B. cereus* phage to 1.0 mg/ml caused a 47 per cent decrease in phage titer; to 0.5 mg/ml, a 42 per cent decrease; to 0.25 mg/ml, a 24 per cent decrease; to 0.1 mg/ml, a 13 per cent decrease; with 0.01 mg/ml no change in titer was found. Phagocidal action of nybomycin on the *B. cereus* phage may be doubtful, since 0.5 μg/ml of nybomycin exerted an antiphage action in the serial dilution tests.

Antibacterial Action.—In the tests for antiphage activity by the paper disk method, inhibition of the host bacteria was observed in some instances. There was, however, no necessary correlation between antiphage and antibacterial activity. For example, antiphage effects were observed for each of the nine staphylophages tested by the paper disk method, but antibacterial activity was noted for *Staphylococcus aureus* 14 and S3K only.

Tests for antibacterial action (bacteria in absence of phage) were made with paper disks dipped in a 0.1 per cent alcoholic solution of nybomycin and by serial dilution[4] in which the compound was suspended in 0.5 per cent CMC or dissolved in hot alcohol and diluted before testing. The disk and dilution tests were substantially in agreement (Table 1). The minimum inhibitory concentrations obtained with the alcoholic solution of nybomycin (figures in parentheses, Table 1) were generally higher than those found when the compound was suspended in CMC.

TABLE 1

ANTIBACTERIAL ACTIVITY OF NYBOMYCIN AS DETERMINED BY PAPER DISK TESTS AND BY SERIAL DILUTION

	Disk Test	Serial Dilution (Minimum Inhibiting Concentration) (μg./ml.)
Staphylococcus aureus, Heatley	−	>50.0
S. aureus No. 11	−	>20.0
S. aureus No. 14	+	1.0
S. aureus S3K	±	5.0
S. muscae	−	>20.0
Escherichia coli (ATCC 9637)	−	>50.0
E. coli Yoeman	−	(>2.5)*
E. coli L-2	−	(>2.5)
E. coli C	−	(>2.5)
E. coli FCb	+	(2.5)
E. coli alpha	−	(>2.5)
E. coli B	+	(0.08)
Bacillus subtilis (ATCC 9633)	+	0.025 (0.25)
B. cereus No. 1	+	2.0
B. mycoides (ATCC 9634)	+	0.8
Mycobacterium smegmatis (ATCC 10143)	+	0.2
Klebsiella pneumoniae (ATCC 9997)	+	0.003 (0.08)
Pseudomonas aeruginosa (ATCC 10145)	−	>50.0

* See text.

Nybomycin evidenced considerable specificity in its antibacterial effects. It was ineffective on the strain of *S. aureus*, Heatley strain, used in this laboratory for

routine antibacterial assays, on *S. aureus* No. 11, and on *Staphylococcus muscae*. It inhibited *S. aureus* No. 14 at 1.0 μg/ml and *S. aureus* S3K at 5.0 μg/ml. It had no effect on the strain of *E. coli* (ATCC 9637) used in our routine antibacterial assays or on *E. coli* Yoeman, *E. coli* L-2, *E. coli* C, and *E. coli* alpha. It was highly effective on *E. coli* B and to a lesser degree on *E. coli* FCb. Nybomycin was highly inhibitory for *Klebsiella pneumoniae* (ATCC 9997), *B. subtilis* (ATCC 9633), *Mycobacterium smegmatis* (ATCC 10143), and *B. mycoides* (ATCC 9634); quite inhibitory for *B. cereus* No. 1; and inactive on *Pseudomonas aeruginosa* (ATCC 10145) (Table 1).

Toxicity Tests.—Preliminary toxicity tests in mice demonstrated that nybomycin suspended in peanut oil and given intraperitoneally was tolerated in doses of 250 mg/kg. Activity, as determined by assay against the test organisms, indicated the presence of active material in the feces for twenty-four hours and in the urine for three days.

Summary.—A colorless crystalline antibiotic with a wide range of activity against bacterial viruses and bacteria has been isolated from an unidentified streptomycete. Methods of production and isolation and some of the chemical and biological properties are described.

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[1] I. N. Asheshov, F. Strelitz, E. A. Hall and H. Flon, *Antibiotics and Chemotherapy*, **4**, 380–394, 1954.

[2] I. N. Asheshov, F. Strelitz, and E. A. Hall, *Antibiotics and Chemotherapy*, **2**, 366–374, 1952.

[3] Huffman Microanalytical Laboratories, Wheatridge, Colorado.

[4] F. Kavanagh, *Bull. Torrey Botan. Club*, **74**, 303–319, 1947.

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*THE SYNERGISM BETWEEN ULTRAVIOLET LIGHT AND TERTIARY-BUTYL HYDROPEROXIDE IN THEIR MUTAGENIC EFFECTIVENESS IN DROSOPHILA**

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After evidence of the mutagenic effect of organic peroxides on bacteria had been obtained by Wyss, Clark, Haas, and Stone[1] in 1948, it was shown by Dickey, Cleland, and Lotz[2] (1948) that tertiary-butyl hydroperoxide acts as a mutagen in *Neurospora crassa* and by L. S. Altenburg[3] (1954) that it is mutagenic when applied as a vapor to the polar cap cells of the early germ track of *Drosophila melanogaster*. Rapoport[4] had also reported, in 1943, evidence of the mutagenicity of oxidizing

substances in *Drosophila* administered by feeding. As pointed out by a number of investigators, the mutagenic action of peroxides is probably based, in part at least, on their characteristic decomposition to form free radicals. Since it has been established that the photochemical decomposition by ultraviolet light of a number of organic peroxides proceeds by way of a free radical mechanism, a test of the mutagenic effectiveness of a combination of tertiary-butyl hydroperoxide and ultraviolet light has been made by exposing the polar caps of *Drosophila* eggs to a very slightly mutagenic dose of the peroxide vapor and simultaneously irradiating them with a very low dose of mutagenic ultraviolet light (2537 A). The synergistic action between these mutagenic agents when administered in this fashion is herein reported. In addition, it has been shown that enhancement of mutagenesis does not occur unless the ultraviolet light strikes the polar caps directly, indicating that here the observed synergistic action between the peroxide and ultraviolet light probably occurs within the cell.

Methods.—The treatments described in these experiments were carried out in a glass cylindrical chamber (4.5 cm. in diameter and 9 cm. long), one end of which consisted of a quartz plate which transmitted 80 per cent of the ultraviolet light falling upon it. The other end of the chamber was left open when the treatment consisted of ultraviolet irradiation alone, but it was possible to make the chamber reasonably airtight, when desired, by sealing the open end with a piece of metal foil coated with Vaseline. The method of obtaining and preparing the eggs for treatment and for exposing them to the peroxide vapor has been described previously by L. S. Altenburg.[3]

The polar cap ends of one group of dechorionated fertilized eggs of *D. melanogaster* containing the cells of the early germ track were exposed to irradiation through the quartz plate from a germicidal lamp (over 90 per cent of the output of which in the ultraviolet spectrum is light of wave length 2537 A) at a distance of 180 cm. for a period of 1 minute. A second group of eggs was treated for 1 minute by exposing the polar caps to the vapor of a 0.5 *M* solution of tertiary-butyl hydroperoxide in di-butyl phthalate. A third group of eggs was placed in the peroxide-saturated chamber for 1 minute, and their polar caps simultaneously irradiated through the quartz plate with the germicidal lamp at a distance of 180 cm. for 1 minute. A fourth group of eggs was exposed to ultraviolet irradiation for 1 minute at 180 cm. in the same chamber after it had been cleared of peroxide vapor and had been saturated with the vapor of di-butyl phthalate (the solvent for the peroxide).

In another series of experiments, the polar caps of one group of eggs were exposed for 10 minutes in the chamber saturated with vapor from the peroxide solution. Another group was placed in the chamber with the polar caps facing toward the metal foil and away from the germicidal lamp. The chamber was then irradiated through the quartz plate with the light from the germicidal lamp for 10 minutes at a distance of 180 cm. from the eggs. Still another series was run in the manner just described, except that the treatment time was 1 minute instead of 10 minutes. However, accidental losses greatly reduced the counts from this series, rendering the results hardly significant, and these are not reported.

After each of the above treatments the eggs were allowed to develop in darkness. After the eggs hatched, males carrying speck (*sp*) as a marker in one member of the second pair of autosomes and nonspeck (sp^+) in the homologous chromosome were

selected and tested for the presence of lethal mutations by the *sifter* technique (Muller[5]). Unfortunately, the stocks used for treatment in the present experiments contained a high proportion of pre-existing lethals (not having been made isogenic for almost a year), but there is no difficulty in identifying these pre-existing lethals by the *sifter* technique, since an entire genome, derived from a given egg, is lethal. The standard errors were calculated by a method (Muller[6]) which takes into account the clustering of the mutants, i.e., the number of times any mutation gave rise to a group of mutations of common origin.

Results (*Table 1*).—The mutation rate per tested chromosome for the eggs treated for 1 minute with ultraviolet light alone was 0.8 ± 0.6 per cent; for those treated for 1 minute with the peroxide vapor alone, 0.9 ± 0.4 per cent; for those treated for 1 minute with both the peroxide vapor and ultraviolet, 10.5 ± 3.5 per cent; and for those treated for 1 minute with di-butyl phthalate vapor and ultraviolet light, 1.5 ± 1.2 per cent. Thus the synergistic action of the peroxide and ultraviolet light treatment is apparent, although part of the enhancement may have been due to the presence of the di-butyl phthalate vapor. A very low mutation rate (0.5 ± 0.5 per cent) had previously been obtained by L. S. Altenburg[3] from eggs exposed for from 20 to 30 minutes to di-butyl phthalate vapor alone.

The mutation rate for eggs exposed for 10 minutes to peroxide vapor alone was 5.6 ± 2.5 per cent; for eggs whose polar caps were shielded from irradiation but were exposed to the irradiated peroxide vapor for 10 minutes at 180 cm., 1.3 ± 0.9 per cent. The difference (4.3 ± 2.7 per cent) between the mutation rate for eggs exposed to peroxide vapor alone for 10 minutes and for eggs exposed to irradiated peroxide vapor for 10 minutes is not significant. Nevertheless, the results show that there is definitely no enhancement of the rate when ultraviolet irradiation is added in this manner—that is, when the light does not strike directly the polar cap ends of the eggs.

Discussion.—The rate of 10.5 ± 3.5 per cent for the combined peroxide vapor and ultraviolet light 1-minute treatment is rather high compared with the rate of 5.7 ± 2.2 per cent reported in a previous experiment which was obtained from eggs treated for from 10 to 30 minutes with peroxide vapor alone.[3] It is strikingly high when compared with the mutagenic effects obtained with ultraviolet light alone in previous experiments in this laboratory,[7] this rate being roughly that obtained when a dose approximately 100 times as great as the present dose was applied. Thus the synergism between these two mutagenic agents is definitely proved, although it must be pointed out that part of the increased mutation rate may have been due to the presence of the di-butyl phthalate used as a solvent for the peroxide.

The vapor method of treatment used in these experiments allows the eggs to be exposed to the products of a high dose of ultraviolet light and peroxide vapor without their being subjected directly to the irradiation. However, in these experiments exposure to peroxide vapor alone for 10 minutes gave a higher mutation rate (5.6 ± 2.5 per cent) than did exposure to irradiated peroxide vapor for 10 minutes (1.3 ± 0.9 per cent), indicating, when considered in conjunction with the result of the 1-minute treatments previously discussed, that the ultraviolet light must strike the cell directly if its synergistic action is to manifest itself. Although the decrease in rate just quoted is not significant, possibly because of the large error involved in the testing method employed, nevertheless it is interesting to speculate

TABLE 1

MUTATION RATES RESULTING FROM SIMULTANEOUS TREATMENT OF *D. melanogaster* EGGS WITH TERTIARY-BUTYL HYDROPEROXIDE AND ULTRAVIOLET LIGHT

(Number of F_3 Cultures [Mutants Shown in Parentheses])

2537 A. ULTRAVIOLET LIGHT, (1 MIN., 180 CM.)		TERTIARY-BUTYL HYDROPEROXIDE VAPOR (1 MIN.)		ULTRAVIOLET PLUS PEROXIDE VAPOR (1 MIN., 180 CM.)		ULTRAVIOLET PLUS DIBUTYL PHTHALATE VAPOR (1 MIN., 180 CM.)	
sp.	*sp*$^+$	*sp*	*sp*$^+$	*sp*	*sp*$^+$	*sp*	*sp*$^+$
13	17(17)*	5	8	3(1)	14	12(3)	8
8	6		6(1)	6	11(11)*	3	9
9(1)	9	4	5	16	12(3)		3
6(6)*	15	7	7	12(1)	13(13)*		4
9		9(1)	13	9(2)	14(14)*	4	
	10(10)*	3	2(2)*	5(1)	8(5)	5(5)*	4
6	4(4)*	10(1)	9	5	3	6	6
	8	11	9	3	3(3)*	3	3
4	4	6	5		6	10	9
7	7	11	14	6	3	20	15
	5(1)	10	15	8	4	16	21
3	6(6)*	7	11		5(5)*	5	10
	4	11	11	3(2)	3	3	12
7	13	10(1)	11	11	11	10	12
7	3	11	11	14(1)	11(7)	5	2(2)*
	9	10	9	5	7	7	4
3	1(1)*	11	14		5	9(1)	15
3	14	11	12				3
8	8	10	12	7	5	3	5
	5	11	8		6		
5		7	9	7(2)	13		
7		12	11	5	6(2)		
3	11	7	10	6(1)	8		
10	5	12	12	4(4)*			
		20	19	7			
112(1)	126(1)	226(3)	248(1)	142(11)	125(17)	116(4)	143(0)
Mutation rates							
0.8 ± 0.6%		0.9 ± 0.4%		10.5 ± 3.5%		1.5 ± 1.2%	

TERTIARY-BUTYL HYDROPEROXIDE VAPOR (10 MIN.)		(POLAR CAPS SHIELDED) ULTRAVIOLET PLUS TERTIARY-BUTYL HYDROPEROXIDE VAPOR (10 MIN.)			
		In Air		In Air plus Water Vapor	
sp.	*sp.*$^+$	*sp*	*sp*$^+$	*sp*	*sp*$^+$
3(1)	2(2)*	15	8(8)*		8(1)
16	10	7	7(7)*	9	8
5	13(3)	15(15)*	28	7	7(7)*
3	14(1)		3		8
6		12(3)	6(6)*	9	7
9	3	5	9	4	4(4)*
	3	12	14		5
	5(3)	8	12	3(3)*	
9	10	6(6)*	10		7
4	7	10	25	8	
4		9	14		5
11(11)*	7	5	4(4)*	3(3)*	
9	7(7)*	14	14		7
9(3)	12		9	8	
7	5(5)*	3			5
	3	6	7(7)*		3(1)
3	3(3)*		3	3	5
4	10		8	7	8
	3(1)			6	3
5	11			6	
3	4				
99(4)	115(8)	106(3)	149(0)	59(0)	61(2)
Mutation rates 5.6 ± 2.5%		1.2 ± 1.2%		1.7 ± 1.7%	

* Pre-existing lethals, not included in totals.

that, if the decrease is real, it may simply reflect the photochemical decomposition of the peroxide into nonmutagenic materials. The photolysis of tertiary-butyl hydroperoxide has not been studied, but the photochemical decomposition by 2537–2650 A ultraviolet light at 30 deg C. of a very closely related peroxide (di-tertiary butyl peroxide) has been investigated by Dorfman and Salsburg.[8] The products of its decomposition are methane, ethane, acetone, and tertiary-butyl alcohol, none of which has so far been reported to be mutagenic.

It is possible to conclude from the results of the experiments herein reported that free radicals or other products formed outside the cells by irradiating the peroxide vapor with ultraviolet light are not able, in present material, to increase the mutation rate appreciably. Perhaps they have a very short half-life and deteriorate before they effectively enter the cell. However, when formed within the cell, they might very well be effective.

Summary.—The synergistic action between ultraviolet light and tertiary-butyl hydroperoxide in producing lethal mutations in the second pair of autosomes in *D. melanogaster* has been demonstrated (when the germ cells are treated at the polar cap stage in the development of the egg and lethals are looked for by the *sifter* technique), the mutation rate produced by the combined agents being 10.5 ± 3.5 per cent, as opposed to 0.8 ± 0.6 per cent for the ultraviolet alone and 0.9 ± 0.4 per cent for the peroxide vapor alone (with the particular low doses of each agent used). A small part of the enhancement of mutagenesis may have been due to the presence of di-butyl phthalate, which was used as a solvent for peroxide and which in combination with ultraviolet light, produced a mutation rate of 1.5 ± 1.2 per cent. When the polar caps of eggs were shielded from the direct rays of the ultraviolet lamp but were otherwise exposed to a combined dose of ultraviolet light and tertiary-butyl hydroperoxide equal to 10 times that applied in the first experiments, no increase in mutation rate was obtained over that produced by the peroxide alone. It may be concluded, therefore, that the synergistic action between these two agents in mutagenesis here detected takes place inside the cell.

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[1] O. Wyss, J. B. Clark, F. Haas, and W. S. Stone, *J. Bacteriol.*, **56**, 51–57, 1948.

[2] F. H. Dickey, G. F. Cleland, and Carol Lotz, these PROCEEDINGS, **35**, 581–586, 1948.

[3] Luolin S. Altenburg, these PROCEEDINGS, **40**, 1037–1040, 1954.

[4] J. A. Rapoport, *Zhur. Obshchei Biol.* (in Russian, with English summary), **4**, 65–72, 1943.

[5] H. J. Muller, *Drosphila Information Service*, **35**, 117–118, 1951.

[6] H. J. Muller, *ibid.*, **37**, 608 (with formula as corrected in mimeographed separate), 1952.

[7] H. J. Muller, Luolin S. Altenburg, Helen Unger Meyer, Margaret Edmondson, and Edgar Altenburg, *Heredity*, **8** (Part 2), 158–185, 1954.

[8] L. M. Dorfman and Z. W. Salsburg, *J. Am. Chem. Soc.*, **73**, 255, 1951.

THE SEISMIC BURIED PULSE

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1. *Introduction.*—In this investigation we determine the motion of the surface of a uniform elastic half-space produced by a point pressure pulse situated at a depth H below the surface and varying with time like the Heaviside unit function $H(t)$. The case of a surface pulse was treated in a previous paper,[1] where the formal solution for the buried pulse was also given. If $w(p)$ denotes the operational representation of the vertical component of the displacement at the surface and $q(p)$ the corresponding expression for the horizontal component, then [2]

$$w(p) = \frac{Zk}{2\pi\mu}\int_0^\infty J_0(\xi r)\xi[-(2\xi^2+k^2)e^{-k\alpha H}+2\xi^2 e^{-k\beta H}]\left(\frac{\alpha}{M}\right)d\xi, \tag{1}$$

$$q(p) = -\frac{Z}{2\pi\mu}\int_0^\infty J_1(\xi r)\xi^2\,[-2k^2\alpha\beta e^{-k\alpha H}+(2\xi^2+k^2)e^{-k\beta H}]\left(\frac{1}{M}\right)d\xi, \tag{2}$$

where

$$M = [(2\xi^2+k^2)^2-4k^2\xi^2\alpha\beta], \tag{3}$$

$$k\alpha = \sqrt{\xi^2+h^2}, \qquad k\beta = \sqrt{\xi^2+k^2}, \qquad k = \frac{p}{c}, \qquad c = \sqrt{\frac{\mu}{\rho}}, \tag{4}$$

$$h = \frac{p}{c_p}, \qquad c_p = \sqrt{\frac{\lambda+2\mu}{\rho}} = \sqrt{3}c. \tag{5}$$

In these equations λ and μ denote the elastic constants of the medium, which are assumed to be equal, ρ is the density, c the shear wave velocity, and c_p the compressional wave velocity. The difference in the normal stress p_{zz} below and above the source is taken to be zero everywhere in the plane of the source but to become infinite at the source in such a manner that its integral over the plane is finite and is equal to Z (negative).

2. *The Vertical Displacement.*—Expression (1) simplifies by the substitution $\xi = kx$ to

$$w(p) = \frac{Zk}{2\pi\mu}\int_0^\infty \frac{J_0(krx)x[-(2x^2+1)e^{-k\alpha H}+2x^2e^{-k\beta H}]\alpha\,dx}{[(2x^2+1)^2-4x^2\,\alpha\beta]}, \tag{6}$$

$$\alpha = \sqrt{x^2+\frac{1}{3}}, \qquad \beta = \sqrt{x^2+1}, \tag{7}$$

where now the operator k appears not only in $kJ_0(krx)$ but also in $e^{-k\alpha H}$ and $e^{-k\beta H}$.

As in Paper I, we seek to transform equation (6) to the form $\int \exp\,[-kf(r, x)]g(x)\,dx$, where the integral is to be taken along such a path in the complex x-plane that along it $f(r, x)$ *is real*, thus leading to the interpretation $\int H[t-(1/c)f(r, x)]g(x)\,dx$. For the case $H = 0$ treated in Paper I, such a path is the imaginary axis v of the x-plane, but this path is no longer suited to our case because

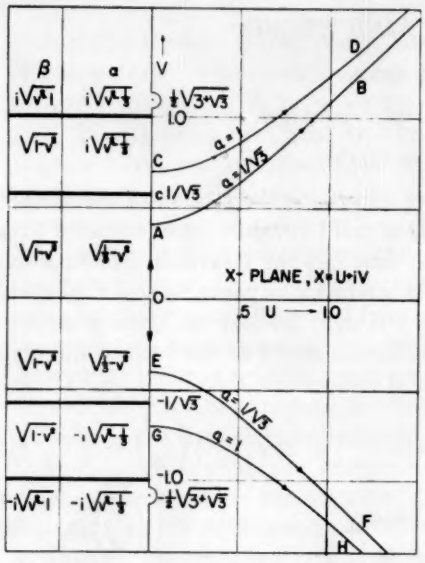

FIG. 1.—The complex x-plane is cut up by branch lines (*thick*) at $x = \pm i/\sqrt{3}$, $\pm i$. The poles are situated at $x = \pm(i/2)\cdot\sqrt{3 + \sqrt{3}}$. The paths chosen for the operational interpretation of $T(p)$ in equation (8) and $S(p)$ in equation (67) are OAB for the case $a = 1/\sqrt{3}$ and OCD for the case $a = 1$.

α and β in the exponents of equation (6) become imaginary for $v > 1/\sqrt{3}$ and $v > 1$, respectively.

2.1. *Method of Solution.*— Let

$$T(p) = \int_0^\infty J_0(krx) f(x) x e^{-kH\sqrt{x^2+a^2}}\, dx, \qquad k = \frac{p}{c}, \quad (8)$$

where a is either $1/\sqrt{3}$ or 1 and where $f(x)$ is an even function of x which has branch points at $x = \pm i/\sqrt{3}$ and at $x = \pm i$. We write

$$T = -\frac{i}{\pi}\int_0^\infty f(x) x e^{-kH\sqrt{x^2+a^2}}\, dx \times \left[\int_0^\infty \exp\,(ikrx\cosh\varphi)\, d\varphi - \int_0^\infty exp\,(-ikrx\cosh\varphi)\, d\varphi\right], \quad (9)$$

and treating, in the first instance, the first φ integral only, we seek to determine a path in the plane $x = u + iv$ such that along it

$$I[H\sqrt{x^2 + a^2} - irx\cosh\varphi] = 0, \quad (10)$$

where I denotes imaginary part. By rationalizing equation (10), one easily finds that such a path is given either by $u = 0$ or by

$$v = (v_0^2 + \rho^2\cosh^2\varphi u^2)^{1/2}, \quad (11)$$

with

$$\rho = \frac{r}{H}, \qquad v_0^2 = \frac{a^2\rho^2\cosh^2\varphi}{1 + \rho^2\cosh^2\varphi}. \quad (12)$$

The portion $u = 0$ is to be followed only for $0 < v < v_0$, and from there on the path is to be taken along the curve (11). This is illustrated in Figure 1 for the case $\rho\cosh\varphi = 1$, where the path OAB applies to the case $a = 1/\sqrt{3}$ and the path OCD to the case $a = 1$. Along the path (11) we have

$$\sqrt{x^2 + a^2} = \frac{v}{\rho\cosh\varphi} + i\rho\cosh\varphi, \quad (13)$$

$$H\sqrt{x^2 + a^2} - irx \cosh \varphi = Hv\left(\rho \cosh \varphi + \frac{1}{\rho \cosh \varphi}\right), \tag{14}$$

$$x = \frac{\sqrt{v^2 - v_0^2}}{\rho \cosh \varphi} + iv. \tag{15}$$

For the part of T involving the second integral in the brackets in equation (9), the paths to be chosen are OEF of Figure 1, or OCH. It is clear that this part of T is the complex conjugate of the first part. Hence

$$T = \frac{2}{\pi} I \int_0^\infty f(x) x e^{-kH\sqrt{x^2+a^2}}\, dx \int_0^\infty \exp(ikrx \cosh \varphi)\, d\varphi = T_1 + T_2, \tag{16}$$

$$T_1 = \frac{2}{\pi} I \int_0^\infty d\varphi \int_{v_0}^\infty \left(\frac{\sqrt{v^2 - v_0^2}}{\rho \operatorname{ch} \varphi} + iv\right)\left(\frac{v}{\rho \operatorname{ch} \varphi \sqrt{v^2 - v_0^2}} + i\right) \times$$
$$f\left(\frac{\sqrt{v^2 - v_0^2}}{\rho \operatorname{ch} \varphi} + iv\right) \exp\left[-kHv\left(\rho \operatorname{ch} \varphi + \frac{1}{\rho \operatorname{ch} \varphi}\right)\right] dv, \tag{17}$$

$$T_2 = -\frac{2}{\pi} I \int_0^\infty d\varphi \int_0^{v_0} f(iv) \exp\left[-kH\sqrt{a^2 - v^2} - krv \operatorname{ch} \varphi\right] v\, dv, \tag{18}$$

where we have used the abbreviation "ch φ" for "cosh φ."

Before proceeding with the operational interpretation of equation (17), it will be convenient to change from the variables φ and v to ψ and y defined by

$$v = v_0 y, \qquad \operatorname{ch} \psi = \frac{\sqrt{1 + \rho^2 \operatorname{ch}^2 \varphi}}{\sqrt{1 + \rho^2}}, \tag{19}$$

whereby equation (17) becomes

$$T_1(p) = \frac{2a}{\pi} I \int_0^\infty v_0(\psi)\, d\psi \int_1^\infty dy \left(\frac{\sqrt{y^2 - 1}}{\rho \operatorname{ch} \varphi} + iy\right)\left(\frac{y}{\rho \operatorname{ch} \varphi \sqrt{y^2 - 1}} + i\right) \times$$
$$f\left(\frac{v_0\sqrt{y^2 - 1}}{\rho \operatorname{ch} \varphi} + iv_0 y\right) \exp(-kaRy \operatorname{ch} \psi), \tag{20}$$

with

$$R = \sqrt{r^2 + H^2}, \qquad h = \frac{H}{R}, \qquad v_0(\psi) = \frac{a}{\operatorname{ch} \psi} \cdot \sqrt{\operatorname{ch}^2 \psi - h^2}. \tag{21}$$

Since k $(= p/c)$ appears only in the exponential, the interpretation of $T_1(p)$ is immediate:

$$T_1(\theta) = \frac{2a}{\pi} I \int_1^\theta dy \int_0^{\operatorname{ch}^{-1}(\theta/y)} v_0(\psi)\, d\psi \left(\frac{h\sqrt{y^2 - 1}}{\sqrt{\operatorname{ch}^2 \psi - h^2}} + iy\right) \times$$
$$\left(\frac{hy}{\sqrt{y^2 - 1}\sqrt{\operatorname{ch}^2 \psi - h^2}} + i\right) f\left[\frac{ah\sqrt{y^2 - 1}}{\operatorname{ch} \psi} + iv_0(\psi) y\right], \tag{22}$$

$$\theta = \frac{\tau}{a}, \qquad \tau = \frac{ct}{R}, \tag{23}$$

for $\theta > 1$, and $T_1(\theta) = 0$, for $\theta < 1$. By differentiating T_1 with respect to t, we get

$$kT_1(\theta) = \frac{2a}{\pi R\theta} I \int_1^{\theta} \frac{y\,dy}{\sqrt{\theta^2 - y^2}} \left[(h\sqrt{y^2 - 1} + i\sqrt{\theta^2 - h^2y^2}) \times \left(\frac{hy^2}{\sqrt{y^2 - 1}\sqrt{\theta^2 - h^2y^2}} + i \right) \right] f(x), \tag{24}$$

where

$$x(y) = \frac{ay}{\theta}(h\sqrt{y^2 - 1} + i\sqrt{\theta^2 - h^2y^2}). \tag{25}$$

With the definition of $x(y)$ given in equation (25), one readily finds that the expression in brackets in equation (24) is equal to $(i\theta/a)\,(dx/dy)$, so that

$$kT_1(\theta) = \frac{2}{\pi R} I \int_1^{\theta} \frac{y\,dy}{\sqrt{\theta^2 - y^2}} \left(i\frac{dx}{dy} \right) f(x) = \frac{2}{\pi R} Re \int_{x(1)}^{x(\theta)} \frac{yf(x)}{\sqrt{\theta^2 - y^2}}\,dx, \tag{26}$$

where

$$x(1) = \frac{ia}{\theta}\sqrt{\theta^2 - h^2}, \qquad x(\theta) = [ah\sqrt{\theta^2 - 1} + ia\theta\sqrt{1 - h^2}] \tag{27}$$

and Re denotes real part. Equation (26) can be further simplified by solving equation (25) for y:

$$\frac{y}{\sqrt{\theta^2 - y^2}} = -\frac{ix}{\sqrt{K(x)}}, \quad K(x) = [x^2 + a^2(h^2 + \theta^2) - 2ah\theta\sqrt{a^2 + x^2}], \tag{28}$$

leading to

$$kT_1(\theta) = 0, \qquad \theta < 1,$$

$$kT_1(\theta) = \frac{2}{\pi R} I \int_{x(1)}^{x(\theta)} \frac{xf(x)\,dx}{\sqrt{K(x)}} = \frac{2}{\pi R} I \int_{s(1)}^{s(\theta)} \frac{sf(\sqrt{s^2 - a^2})\,ds}{\sqrt{K(s)}}, \qquad \theta > 1, \tag{29}$$

where

$$s = \sqrt{x^2 + a^2}, \qquad s(1) = \frac{ah}{\theta}, \qquad s(\theta) = [ah\theta + ia\sqrt{\theta^2 - 1}\sqrt{1 - h^2}], \tag{30}$$

$$K(s) = [(s - ah\theta)^2 + a^2(1 - h^2)(\theta^2 - 1)]. \tag{31}$$

The interpretation of equation (18) leads directly to

$$T_2 = -\frac{2}{\pi} I \int_0^{\infty} d\varphi \int_0^{v_0} f(iv) H[t - \frac{1}{c}(rv\,\mathrm{ch}\,\varphi + H\sqrt{a^2 - v^2})]\,v\,dv, \tag{32}$$

requiring that

$$\frac{a\theta}{h} = \frac{ct}{H} > [v\rho \operatorname{ch} \varphi + \sqrt{a^2 - v^2}] \equiv m(v). \tag{33}$$

Now $m(v)$ is a monotone-increasing function of v in the whole range $0 < v < v_0$, reaching a maximum value at v_0 of

$$m(v_0) = a\sqrt{1 + \rho^2 \operatorname{ch}^2 \varphi}. \tag{34}$$

If $\theta > 1$, then for $(ct/H) > a\sqrt{1 + \rho^2 \operatorname{ch}^2 \varphi}$, v may cover the whole range, provided that $0 < \varphi < \omega$, while for $(ct/H) < a\sqrt{1 + \rho^2 \operatorname{ch}^2 \varphi}$, v must be less than ν, and $\varphi > \omega$, where

$$\operatorname{ch} \omega = \frac{1}{\rho}\sqrt{\theta^2 - 1}, \qquad \nu = \frac{1}{1 + \rho^2 \operatorname{ch}^2 \varphi}\left[\frac{ct}{H}\rho \operatorname{ch} \varphi - \sqrt{a^2(1 + \rho^2 \operatorname{ch}^2 \varphi) - (ct/H)^2}\right]. \tag{35}$$

If $\theta < 1$, then $0 < v < \nu$, and φ is unrestricted. Hence

$$T_2 = 0, \qquad \theta < h,$$

$$T_2 = -\frac{2}{\pi} I \int_0^\infty d\varphi \int_0^\nu f(iv) v\, dv, \qquad h < \theta < 1,$$

$$T_2 = -\frac{2}{\pi} I \int_0^\omega d\varphi \int_0^{v_0} f(iv) v\, dv - \frac{2}{\pi} I \int_\omega^\infty d\varphi \int_0^\nu f(iv) v\, dv, \qquad \theta > 1. \tag{36}$$

On differentiating equation (36) now with respect to t, we get

$$kT_2 = -\frac{2}{\pi c} I \int_0^\infty d\varphi \left(\frac{\partial \nu}{\partial t}\right) f(i\nu)\nu, \qquad h < \theta < 1,$$

$$kT = -\frac{2}{\pi c} I \int_\omega^\infty d\varphi \left(\frac{\partial \nu}{\partial t}\right) f(i\nu)\nu, \qquad \theta > 1. \tag{37}$$

Here we change the variable of integration to ν. Since, when $v = \nu$,

$$\rho \operatorname{ch} \varphi = \frac{ct}{H\nu} - \frac{1}{\nu}\sqrt{a^2 - \nu^2}, \tag{38}$$

we obtain from equations (35) and (38) the relation

$$\frac{\partial \varphi}{\partial \nu} \cdot \frac{\partial \nu}{\partial t} = -\frac{c}{R\sqrt{L(v)}}, \tag{39}$$

$$L(v) = K(ix) = [a^2(h^2 + \theta^2) - v^2 - 2ah\theta\sqrt{a^2 - v^2}], \tag{40}$$

leading to

$$kT_2 = -\frac{2}{\pi R} I \int_0^\epsilon \frac{f(iv) v\, dv}{\sqrt{L(v)}} = \frac{2}{\pi R} I \int_0^{i\epsilon} \frac{f(x) x\, dx}{\sqrt{K(x)}}, \qquad h < \theta < 1,$$

$$kT_2 = -\frac{2}{\pi R} I \int_0^\omega \frac{f(iv) v\, dv}{\sqrt{L(v)}} = \frac{2}{\pi R} I \int_0^{x(1)} \frac{f(x) x\, dx}{\sqrt{K(x)}}, \qquad \theta > 1, \tag{41}$$

where we have reverted from ν to v, and

$$\epsilon(\theta) = a\theta\sqrt{1-h^2} - ah\sqrt{1-\theta^2}, \tag{42}$$

$$\omega(\theta) = \frac{a}{\theta}\sqrt{\theta^2-h^2}. \tag{43}$$

Substituting equations (29) and (41) in equation (16), we get

$$\begin{aligned} kT &= k\int_0^\infty J_0(krx)f(x)xe^{-kH\sqrt{x^2+a^2}}dx = 0, && \theta < h, \\ &= -\frac{2}{\pi R}I\int_0^{\epsilon(\theta)}\frac{f(iv)v\,dv}{\sqrt{L(v)}} = \frac{2}{\pi R}I\int_0^{i\epsilon(\theta)}\frac{f(x)x\,dx}{\sqrt{K(x)}}, && h < \theta < 1, \quad (44) \\ &= -\frac{2}{\pi R}I\int_0^{v(\theta)}\frac{f(iv)v\,dv}{\sqrt{L(v)}} = \frac{2}{\pi R}I\int_0^{x(\theta)}\frac{f(x)x\,dx}{\sqrt{K(x)}} = \\ &\qquad \frac{2}{\pi R}I\int_a^{s(\theta)}\frac{sds\,f(\sqrt{s^2-a^2})}{\sqrt{K(s)}}, && \theta > 1, \quad (45) \end{aligned}$$

with

$$v(\theta) = a\theta\sqrt{1-h^2} - iah\sqrt{\theta^2-1}, \quad x(\theta) = iv(\theta). \tag{46}$$

2.2. *The Case* $a = 1/\sqrt{3}$.—In the case in which the value of a in the exponent of equation (16) is equal to $1/\sqrt{3}$, the integral T_2 in equation (18) vanishes, because, along the portion OA of Figure 1, $If(iv) = 0$. Equation (29) then gives kT, with

$$\theta = \sqrt{3}\tau, \qquad s(1) = \frac{h}{3\tau}, \qquad s(\theta) = h\tau + iv_0, \tag{47}$$

$$v_0 = \sqrt{1-h^2}\sqrt{\tau^2-\frac{1}{3}}, \qquad K(s) = [(s-h\tau)^2 + v_0^2], \tag{48}$$

$$kT\left(\frac{1}{\sqrt{3}}\right) = \frac{2}{\pi R}I\int_{s(1)}^{s(\theta)}\frac{sf\left(\sqrt{s^2-\frac{1}{3}}\right)ds}{\sqrt{K(s)}} = \frac{2}{\pi R}I\int_{h\tau}^{s(\theta)}\frac{sf\left(\sqrt{s^2-\frac{1}{3}}\right)ds}{\sqrt{K(s)}}, \tag{49}$$

since the integrand is real between $s(1)$ and $h\tau$.

For the purpose of numerical evaluation of the integral, it is convenient to substitute

$$s = h\tau + iv_0\sin\varphi, \tag{50}$$

leading to the result that, under the physical conditions of our problem,

$$kT\left(\frac{1}{\sqrt{3}}\right) = k\int_0^\infty J_0(krx)f(x)x\exp\left(-kH\sqrt{x^2+\frac{1}{3}}\right)dx \to 0, \quad \tau < \frac{1}{\sqrt{3}}, \tag{51}$$

$$\to \left(\frac{2}{\pi R}\right)\mathrm{Re}\int_0^{\pi/2}(h\tau + i\nu_0\sin\varphi)f\left[\sqrt{(h\tau+i\nu_0\sin\varphi)^2-\frac{1}{3}}\right]d\varphi,$$

$$\tau > \frac{1}{\sqrt{3}}. \tag{52}$$

2.3. *The Case $a = 1$.*—When $a = 1$, we must use equations (44) and (45), since T_2 does not vanish, $If(iv)$ not being zero along the portion cC of the path in Figure 1. Here we have

$$\theta = \tau, \qquad \epsilon(\tau) = [\tau\sqrt{1-h^2} - h\sqrt{1-\tau^2}]. \tag{53}$$

For $\tau < 1$, we can write equation (44) as

$$kT = -\frac{2}{\pi R}I\int_{1/\sqrt{3}}^{\epsilon(\tau)} f(iv)\left[\frac{v}{\sqrt{L(v)}}\right]dv, \qquad h < \tau < 1, \tag{54}$$

since $If(iv) = 0$ for $0 < v < 1/\sqrt{3}$. kT will be different from zero only if $\epsilon(\tau) > 1/\sqrt{3}$. Now $\epsilon(\tau)$ starts with the value 0 at $\tau = h$ and increases in a monotone fashion to its maximum value of $\sqrt{1-h^2}$ at $\tau = 1$. Hence kT is zero unless

$$\sqrt{1-h^2} > \frac{1}{\sqrt{3}}, \qquad h < \sqrt{2/3}, \qquad r > \frac{H}{\sqrt{2}}. \tag{55}$$

When $h < \sqrt{2/3}$, the condition $\epsilon(\tau) > 1/\sqrt{3}$ requires that $\tau > \tau^*$, where

$$\tau^* = \left(\sqrt{2/3}\,h + \frac{1}{\sqrt{3}}\sqrt{1-h^2}\right) < 1. \tag{56}$$

It follows that

$$kT(1) = k\int_0^\infty J_0(krx)f(x)x\exp\left(-kH\sqrt{x^2+1}\right)dx \to 0, \qquad r < \frac{H}{\sqrt{2}}, \tau < 1, \tag{57}$$

$$\to -\frac{2}{\pi R}I\int_{1/\sqrt{3}}^{\epsilon(\tau)} f(iv)\left[\frac{v}{\sqrt{L(v)}}\right]dv, \qquad r > \frac{H}{\sqrt{2}}, \tau^* < \tau < 1. \tag{58}$$

The physical significance of conditions (55) and (56) becomes clear when we realize that $a = 1$ refers to the SV-wave excited by the source and that $\tau < 1$ refers to events which happen before the time of arrival of the direct S-wave. Now, when the SV-wave reaches the surface, it is reflected partly as an SV-wave, and partly as a P-wave, for epicentral distances r less than $H/\sqrt{2}$, as is shown in Figure 2, *a*. When $r = H/\sqrt{2}$ (Fig. 2, *b*), the incident S-wave is totally reflected, and a diffracted P'-wave is directed in a horizontal direction. At a point B in Figure 2, *c*, situated beyond $H/\sqrt{2}$, the reflected wave S' is of the same amplitude as the incident S, but with a different phase, and there is no reflected P'-wave. However,

before the arrival of the direct S-wave, the point B receives a diffracted P'-wave which reaches it by the path OAB, of which the leg OA is traversed with the shear wave velocity c and the leg AB with the velocity of compressional waves $\sqrt{3}c$. The travel time of this refracted (diffracted) wave is given by

$$ct = \left[\sqrt{2/3}\, H + \frac{r}{\sqrt{3}}\right], \tag{59}$$

corresponding precisely to $\tau = \tau^*$ in equation (56). The integral (58) therefore represents the diffracted P-wave derived from the SV-wave incident on the surface.

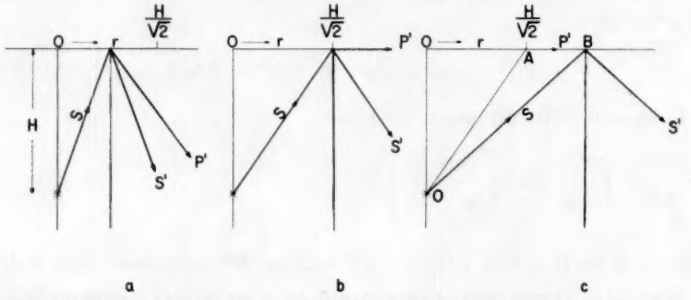

FIG. 2.—Ray diagram of the reflection of an SV-wave at a free surface. S' denotes incident SV-wave, S' the reflected shear wave, and P' the derived compressional wave. In diagram a, $r < H/\sqrt{2}$. In diagram b, S is totally reflected, and a diffracted P' travels along the surface. In diagram c, $(r > H/\sqrt{2})$, the point B receives, before the arrival of the direct S-wave, a diffracted P'-wave which traveled along OA with the shear velocity c and along AB with the compressional velocity $\sqrt{3}c$. The diagram was drawn under the assumption that the elastic constants λ and μ are equal.

When $\tau > 1$, i.e., after the arrival of the direct S-wave, one can easily show that in the case $h > \sqrt{2/3}$

$$I \int_a^{h\tau} sf(\sqrt{s^2 - 1}) \left[\frac{1}{\sqrt{K(s)}}\right] ds = 0, \tag{60}$$

because the integrand is real. Hence, from equation (45),

$$\begin{aligned} kT(1) &= \frac{2}{\pi R} I \int_{h\tau}^{s(\tau)} \frac{sf(\sqrt{s^2 - 1})\, ds}{\sqrt{K(s)}} \\ &= \frac{2}{\pi R} Re \int_0^{\pi/2} (h\tau + i\mu_0 \sin\varphi) f[\sqrt{(h\tau + i\mu_0 \sin\varphi)^2 - 1}]\, d\varphi, \\ & \qquad\qquad r < \frac{H}{\sqrt{2}}, \quad \tau > 1, \end{aligned} \tag{61}$$

where we have used the substitution

$$s = h\tau + i\mu_0 \sin\varphi, \qquad \mu_0 = \sqrt{(1 - h^2)(\tau^2 - 1)}. \tag{62}$$

When $r > (H/\sqrt{2})$, equation (60) is not always satisfied, and kT takes on the following form:

$$kT(1) = \int_0^\infty J_0(krx)f(x) \exp(-kH\sqrt{x^2+1})x\,dx$$

$$\to \frac{2}{\pi R} Re \int_0^{\pi/2} d\varphi(h\tau + i\mu_0 \sin\varphi) f\left[\sqrt{(h\tau + i\mu_0 \sin\varphi)^2 - 1}\right] d\varphi -$$

$$\frac{2}{\pi R} I \int_{1/\sqrt{3}}^{\sqrt{1-h^2\tau^2}} \frac{f(iv)v\,dv}{\sqrt{L(v)}}, \qquad 1 < \tau < \frac{\sqrt[2]{3}}{h}, r > \frac{H}{\sqrt{2}}, \quad (63)$$

$$\to \frac{2}{\pi R} Re \int_0^{\pi/2} (h\tau + i\mu_0 \sin\varphi) f\left[\sqrt{(h\tau + i\mu_0 \sin\varphi)^2 - 1}\right] d\varphi,$$

$$\tau > \frac{\sqrt[2]{3}}{h}, \qquad r > \frac{H}{\sqrt{2}}. \quad (64)$$

Equations (51), (52), (57), (58), (63), and (64) enable us to determine the vertical component of the displacement $w(t)$ from the operational representation $w(p)$ given in equation (6).

3. *The Horizontal Displacement.*—By the substitution $\xi = kx$ in equation (2), the expression $q(p)$ for the operational representation of the horizontal displacement at the surface becomes

$$q(p) = -\frac{Zk}{2\pi\mu} \int_0^\infty \frac{J_1(kxr)x^2[-2\alpha\beta e^{-kH\alpha} + (2x^2+1)e^{-kH\beta}]\,dx}{[(2x^2+1)^2 - 4x^2\alpha\beta]}, \quad (65)$$

$$\alpha = \sqrt{x^2 + {}^1/_3}, \qquad \beta = \sqrt{x^2+1}. \quad (66)$$

As in Section 2, we consider the function

$$S(p) = -k \int_0^\infty J_1(krx)x^2 g(x) e^{-kH\sqrt{x^2+a^2}}\,dx, \qquad k = \frac{p}{c}, \quad (67)$$

where a is either $(1/\sqrt{3})$ or 1 and where $g(x)$ is an even function of x having branch points at $x = \pm i/\sqrt{3}$ and at $x = \pm i$. S can be written as

$$S = \frac{2k}{\pi} \int_0^\infty x^2 g(x) e^{-kH\sqrt{x^2+a^2}}\,dx \int_0^\infty \operatorname{ch}\varphi \cos(kxr \operatorname{ch}\varphi)\,d\varphi$$

$$= \frac{2k}{\pi} Re \int_0^\infty x^2 g(x) e^{-kH\sqrt{x^2+a^2}}\,dx \int_0^\infty \operatorname{ch}\varphi \exp(ikxr \operatorname{ch}\varphi)\,d\varphi, \quad (68)$$

which is similar in form to T in equation (16). The interpretation of equation (68) can be carried out following the method used for T, the only difference consisting in the appearance of the extra factor $x \operatorname{ch}\varphi$ in the integrand of S. By equation (38), this adds the following factor in the integrand of S_2:

$$x \operatorname{ch}\varphi = i\nu \operatorname{ch}\varphi = \frac{i}{\sqrt{1-h^2}}(a\theta - h\sqrt{a^2 - \nu^2}), \quad (69)$$

and the same extra factor appears in the integrand of S_1.
The result is

$$S = -k\int_0^\infty J_1(kxr)x^2g(x)e^{-kH\sqrt{x^2+a^2}}\,dx \to 0 \qquad \theta < h,$$

$$\to \frac{2}{\pi r} I\int_0^{v(\theta)} (a\tau - h\sqrt{a^2 - v^2})g(iv)\left[\frac{v}{\sqrt{L(v)}}\right]dv, \qquad h < \theta < 1, \quad (70)$$

$$\to \frac{2}{\pi r} I\int_0^{v(\theta)} (a\tau - h\sqrt{a^2 - v^2})g(iv)\left[\frac{v}{\sqrt{L(v)}}\right]dv, \qquad \theta > 1. \quad (71)$$

Putting $a = 1/\sqrt{3}$, we get

$$S\left(\frac{1}{\sqrt{3}}\right) = -k\int_0^\infty J_1(kxr)x^2g(x)e^{-kH\sqrt{x^2+1/3}} \to 0, \qquad \tau < \frac{1}{\sqrt{3}},$$

$$\to -\frac{2}{\pi r} Re\int_0^{\pi/2} d\varphi(\tau - h^2\tau - ih\nu_0 \sin\varphi)\,(h\tau + i\nu_0 \sin\varphi)\times$$

$$g\left[\sqrt{(h\tau + i\nu_0 \sin\varphi)^2 - 1/3}\right], \qquad \tau > \frac{1}{\sqrt{3}}. \quad (72)$$

Similarly,

$$S(1) = -k\int_0^\infty J_1(kxr)x^2g(x)e^{-kH\sqrt{x^2+1}} \to 0, \qquad \tau < 1, r < \frac{H}{\sqrt{2}},$$

$$\to -\frac{2}{\pi r} Re\int_0^{\pi/2} d\varphi(\tau - h^2\tau - ih\mu_0 \sin\varphi)\,(h\tau + i\mu_0 \sin\varphi)g\times$$

$$\left[\sqrt{(h\tau + i\mu_0 \sin\varphi)^2 - 1}\right], \tau > 1, r < \frac{H}{\sqrt{2}}, \quad (73)$$

while for the case $r > H/\sqrt{2}$ we get

$$S(1) = -k\int_0^\infty J_1(kxr)x^2g(x)e^{-kH\sqrt{x^2+1}} \to 0 \qquad \tau < \tau^*$$

$$\to \frac{2}{\pi r} I\int_{1/\sqrt{3}}^{\epsilon(\tau)} (\tau - h\sqrt{1 - v^2})g(iv)\left[\frac{v}{\sqrt{L(v)}}\right]dv, \qquad \tau^* < \tau < 1, \quad (74)$$

$$\to -\frac{2}{\pi r} Re\int_0^{\pi/2} d\varphi(\tau - h^2\tau - ih\mu_0 \sin\varphi)\,(h\tau + i\mu_0 \sin\varphi)\times$$

$$g\left[\sqrt{(h\tau + i\mu_0 \sin\varphi)^2 - 1)}\right] +$$

$$\frac{2}{\pi r} I\int_{1/\sqrt{3}}^{\sqrt{1-h^2\tau^2}} (\tau - h\sqrt{1 - v^2})g(iv)\left[\frac{v}{\sqrt{L(v)}}\right]dv, \qquad 1 < \tau < \frac{\sqrt{2/3}}{h}, \quad (75)$$

$$\to -\frac{2}{\pi r} Re\int_0^{\pi/2} d\varphi(\tau - h^2\tau - ih\mu_0 \sin\varphi)\,(h\tau + i\mu_0 \sin\varphi)\times$$

$$g\left[\sqrt{(h\tau + i\mu_0 \sin\varphi)^2 - 1}\right], \qquad \tau > \frac{\sqrt{2/3}}{h}. \quad (76)$$

With the aid of equations (72) and (76) we can determine the horizontal displacement $q(t)$, using its operational representation given in equation (65).

4. *Summary.*—An exact solution is given for the motion of the surface of a uniform elastic half-space produced by a point pressure pulse situated at a depth H below the surface. The time variation of the pressure pulse is assumed to be given by the Heaviside unit function. The singularity of the point source is specified by the condition that the integral of the difference of the normal stress over a horizontal plane passing through the source is finite. This source excites both SV-waves and P-waves. The operational representation of the vertical component of displacement at the surface is given in equation (6), and its interpretation can be carried out with the aid of equations (51), (52), (57), (58), (63), and (64). The operational representation of the horizontal component of the displacement $q(p)$ is given by equation (65), and its interpretation can be made by using equations (72)–(76). The interpretation is accomplished by transforming the paths of integration in equations (6) and (65) from the real axis to the paths OAB and OCD shown in Figure 1.

The motion of the surface is different in the case $r < H/\sqrt{2}$ from the case $r > H/\sqrt{2}$, where r denotes epicentral distance. When $r > H/\sqrt{2}$ (for a medium in which the elastic constants λ and μ are assumed to be equal), the P-wave is followed by a diffracted P-wave, derived from the SV-wave, which arrives before the direct SV-wave.[3] This ray picture is illustrated in Figure 2. This refracted (diffracted) P-wave is represented in our solution by the integrals (58) and (74).

The numerical evaluation of the motion of the surface due to the buried source for various distances from the source will be given in a subsequent publication.

[1] C. L. Pekeris, these PROCEEDINGS, **41**, 469, 1955. This paper will be referred to as "Paper I." The expression for the vertical displacement in the case of a surface pulse was first given in these PROCEEDINGS, **26**, 433, 1940.

The method followed in this paper is similar to the one used at about the same time by L. Cagniard in *Réflexion et réfraction des ondes seismiques progressives* (Paris: Gauthier-Villars, 1939). I owe this reference to C. H. Dix, *Geophysics*, **19**, 722, 1954. See also E. Pinney, *Bull. Seis. Soc. Amer.*, **44**, 571, 1954.

[2] Equations (20) and (21) in Paper I.

[3] This diffracted wave was found by H. Nakano in his analysis of the case of a buried line source (*Jap. J. Astr. Geophys.*, **2**, 233, 1925). See also E. R. Lapwood, *Phil. Trans. Roy. Soc.*, **A242**, 63, 1949. For observations of this wave in two-dimensional model experiments see F. Press, J. Oliver, and M. Ewing, *Geophysics*, **19**, 391, 1954.

ON HERMITIAN OPERATORS OVER THE CAYLEY ALGEBRA

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Let $\mathfrak{P}$ be the vector space of all n-tuples $X = (x_1, \ldots, x_n)$ with co-ordinates x_i in the Cayley algebra $\mathfrak{C}$ of dimension 8 over the field $\mathfrak{R}$ of all real numbers. Then $\mathfrak{P}$ is a vector space of dimension $8n$ over $\mathfrak{R}$. The algebra $\mathfrak{C}$ has an involution $x \to x^*$, and we define an inner product in $\mathfrak{P}$ by

$$(X, Y) = \sum_{i=1}^{n} x_i y_i^*, \tag{1}$$

for all vectors $X = (x_1, \ldots, x_n)$ and $Y = (y_1, \ldots, y_n)$ in $\mathfrak{L}$. We seek to determine the set $\mathfrak{A}$ of all Hermitian operators T on $\mathfrak{L}$, that is, the set of all linear transformations T over $\mathfrak{R}$ of $\mathfrak{L}$ such that

$$(XT, Y) = (X, YT). \tag{2}$$

It is easy to verify that $\mathfrak{A}$ is a special Jordan algebra over $\mathfrak{R}$. We shall derive the following result.

THEOREM. *The algebra $\mathfrak{A}$ is isomorphic to the special Jordan algebra of all n-rowed real symmetric matrices.*

For, consider the equation

$$(xT)y^* = x(yS)^*, \tag{3}$$

for all x and y of $\mathfrak{C}$, where S and T are linear transformations over $\mathfrak{R}$ of $\mathfrak{C}$. Equation (2) reduces to equation (3), with $S = T$ when $n = 1$. The algebra $\mathfrak{C}$ has a unity element $e = e^*$, and equation (3) implies that

$$t = eT = (eS)^*, \qquad xT = xt, \qquad (yS)^* = ty^*. \tag{4}$$

But then equation (3) is equivalent to

$$(xt)y^* = x(ty^*) \tag{5}$$

for every x and y of $\mathfrak{C}$. It follows that t is in the nucleus of $\mathfrak{C}$, and so $t = \tau e$, where τ is real,

$$T = \tau I = S. \tag{6}$$

We now consider equation (2). The transformation T may be regarded as being an n-rowed square matrix $T = (T_{ij})$ whose elements T_{ij} are linear transformations on $\mathfrak{C}$. Then equation (2) is equivalent to

$$\sum_{i,j=1}^{n} (x_i T_{ij}) y_j^* = \sum_{i,j=1}^{n} x_i (y_j T_{ji})^*. \tag{7}$$

Take $X = (0, \ldots, 0, x_i, 0, \ldots, 0)$, $Y = (0, \ldots, 0, y_j, 0, \ldots, 0)$, and see that equation (7) becomes

$$(x_i T_{ij}) y_j^* = x_i (y_j T_{ji})^*. \tag{8}$$

This is equation (3), with $T = T_{ij}$, $S = T_{ji}$. Then $T = S = \tau_{ij} I = \tau_{ji} I$ by equation (6), and so $T = (\tau_{ij} I)$ is a real symmetric matrix. The converse is trivial, and we have proved our theorem.

*RELATIONS BETWEEN THE COHOMOLOGY GROUPS OF DOLBEAULT AND TOPOLOGICAL INVARIANTS**

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For complex-analytic manifolds there are the invariants of the complex structure on one side and the topological invariants of the underlying manifold on the other. It seems that few relations between them are known, except in the special case of Kähler manifolds.

In this note a spectral sequence is defined which relates the cohomology groups of Dolbeault as invariants of the complex structure to the groups of de Rham as topological invariants (Theorem 3). Two applications are given: the Euler characteristic of the manifold is expressed by means of the dimensions of the Dolbeault groups (Theorem 1); for the Betti numbers, corresponding inequalities are obtained (Theorem 2).

1. Let M be a complex-analytic manifold, m its complex dimension. Ω^q shall denote the sheaf of germs of holomorphic differential forms of degree q, $H^p(M, \Omega^q)$ the pth cohomology group with coefficients in this sheaf.[1]

THEOREM 1. *Between the Euler characteristic χ of M and the dimensions $b_{p,q}$ of the Dolbeault cohomology groups $H^p(M, \Omega^q)$ there holds the equality*

$$\chi = \sum_{p,q=0}^{m} (-1)^{p+q} b_{p,q}. \tag{1}$$

THEOREM 2. *For the Betti number b_r of M the following inequality holds:*

$$b_r \leq \sum_{p+q=r} b_{p,q} \qquad (r = 0, 1, 2, \ldots, 2m). \tag{2}$$

Remark: Using a result of Serre,[2] which states that $b_{p,q} = b_{m-p,m-q}$, one can reduce the number of terms in equality (1) and inequality (2). Hence m odd implies χ even (it is known that χ is even for any manifold of real dimension $4k-2$).

2. Before stating Theorem 3 and giving the outlines of the proofs, it will be useful to state some general facts on spectral sequences. All notations used here are from the appendix of the work cited in note 3. Proofs can be found there[3] or in the original paper.[4] The spectral sequence is a sequence of groups and homomorphisms which can be associated to any K-module T (K denotes a principal ring) which has a decreasing filtration $\{{}^pT\}$ (i.e., a sequence of submodules ${}^pT$ with ${}^{p+1}T \subset {}^pT$), a differential operator d (i.e., an endomorphism of T with $d^2 = 0$), and a gradation $T = \bigoplus_n T^n$ ($\oplus$ denotes weak direct sum), satisfying the following conditions of compatibility: $d(T^n) \subset T^{n+1}$; $d({}^pT) \subset {}^pT$; ${}^pT = \bigoplus_n ({}^pT \cap T^n)$. We put ${}^pT^n = {}^pT \cap T^n$. Let $Z \subset T$ be the kernel of the endomorphism d, B the image $d(T) \subset T$, and $H = Z/B$. The gradation and filtration of T induce gradations and filtrations of Z, B, and H in an evident way. So we have, e.g., ${}^pZ^n = Z \cap {}^pT^n$; ${}^pB^n = B \cap {}^pT^n$; ${}^pH^n = {}^pZ^n/{}^pB^n$. One defines ${}^pT_r^n$ to be the submodule of T, consisting of all elements t of ${}^pT^n$, for which $dt \in {}^{p+r}T$, i.e.,

$${}^pT_r^n = \{t \mid t \in {}^pT^n;\ dt \in {}^{p+r}T\}.$$

Analogous:

$$ {}^pB_r^n = \{t | t \in {}^pT^n;\ t = ds;\ s \in {}^{p-r}T\}. $$

The modules of the spectral sequence are, by definition,

$$ E_r^{p,q} = \frac{{}^pT_r^{p+q}}{{}^{p+1}T_{r-1}^{p+q} + {}^pB_{r-1}^{p+q}}; \qquad E_\infty^{p,q} = \frac{{}^pZ^{p+q}}{{}^{p+1}Z^{p+q} + {}^pB^{p+q}}. $$

We make use of the following general properties of the spectral sequence:

a) The differential operator d induces homomorphisms d_r:

$$ \ldots \to E_r^{p-r,q+r-1} \xrightarrow{d_r} E_r^{p,q} \xrightarrow{d_r} E_r^{p+r,q-r+1} \to \ldots $$

such that $(d_r)^2 = 0$ and the groups $E_{r+1}^{p,q}$ are isomorphic to the "cohomology groups" of the above sequence; i.e., denoting by $K_r^{p,q} \subset E_r^{p,q}$ the d_r-kernel, we have

$$ E_{r+1}^{p,q} \cong \frac{K_r^{p,q}}{d_r(E_r^{p-r,q+r-1})}. \tag{3} $$

b) $E_\infty^{p,q} \cong {}^pH^{p+q}/{}^{p+1}H^{p+q}$.

c) If ${}^sT = 0$ and ${}^0T = T$ (i.e., filtration bounded above by s and below by 0), then we have $E_r^{p,q} = E_\infty^{p,q}$ for $r \geq N$, where $N = \max(s - p, p + 1)$.

3. For the investigation of complex manifolds, now let T be the C-module (C = field of complex numbers) of all complex-valued differential forms on M (assume always of class C^∞). The endomorphism d is the exterior derivation of differential forms; the gradation is given by the ordinary degree of differential forms. The filtration is obtained as follows: It is well known that each differential form φ has a unique decomposition $\varphi = \sum \varphi_{r,s}$ where $\varphi_{r,s}$ is "of type (r, s)," i.e., $\varphi_{r,s} = \sum \varphi_{\alpha_1 \ldots \alpha_r \beta_1 \ldots \beta_s} dz^{\alpha_1} \wedge \ldots dz^{\alpha_r} \wedge d\bar{z}^{\beta_1} \wedge \ldots d\bar{z}^{\beta_s}$, the z^ν being any local complex co-ordinates. Define ${}^pT$ to consist of those forms φ for which in the above decomposition $\varphi_{r,s} = 0$ for $r < p$ ("degree in the $dz^\nu \geq p$). The compatibility conditions are satisfied; hence the spectral sequence is defined. Further, the filtration is obviously bounded above by m and below by 0; hence property *c* holds with $s = m$.

4. Consider now the terms $E_1^{p,q}$ of the spectral sequence:

$$ E_1^{p,q} = \frac{{}^pT_1^{p+q}}{{}^{p+1}T_0^{p+q} + {}^pB_0^{p+q}}. $$

The elements of ${}^pT_1^{p+q}$ are the forms which can be written as $\varphi = \varphi_{p,q} + \varphi_{p+1,q-1} + \ldots$, with $d\varphi \in {}^{p+1}T^{p+q}$. This implies that $\bar{\partial}\varphi_{p,q} = 0$, where $\bar{\partial}$ comes from the well-known decomposition of d: $d = \partial + \bar{\partial}$, ∂ raising the degree in the dz^ν, $\bar{\partial}$ the degree in the $d\bar{z}^\nu$. Since we calculate modulo ${}^{p+1}T^{p+q}$, the other terms of φ, namely, $\varphi_{p+1,q-1}, \varphi_{p+2,q-2}, \ldots$, are of no importance. But, since we calculate also modulo ${}^pB_0^{p+q}$, one easily verifies that $E_1^{p,q}$ is isomorphic to the factor module of those forms $\varphi_{p,q}$ of type (p, q), whose $\bar{\partial}\varphi_{p,q}$ vanishes, modulo those which are the $\bar{\partial}$-image of some form (of type $(p, q-1)$, of course). But, by Dolbeault's theorem, this factor space of the $\bar{\partial}$-closed forms of type (p, q) modulo the $\bar{\partial}$-exact ones is isomorphic to the Dolbeault cohomology group $H^q(M, \Omega^p)$. Hence:

LEMMA 1. $$E_1^{p,q} \cong H^q(M, \Omega^p).$$

Of importance are, further, the terms $E_\infty^{p,q}$ of the spectral sequence, which are, as mentioned in property b, isomorphic to ${}^pH^{p+q}/{}^{p+1}H^{p+q}$. The groups ${}^pH^n$ are the subgroups of the group H^n, induced by the filtration of T. The group H^n is now by definition just the nth de Rham group, which is filtered as follows:

$$H^n = {}^0H^n \supset {}^1H^n \supset {}^2H^n \supset \ldots {}^mH^n \supset {}^{m+1}H^n = 0. \tag{4}$$

So we have

LEMMA 2. *The groups $E_\infty^{p,q}$ are isomorphic to the factor groups ${}^pH^{p+q}/{}^{p+1}H^{p+q}$, where the groups ${}^jH^{p+q}$ ($j = 0, 1, \ldots m+1$) form a filtration of the $(p+q)$th de Rham group.*

One usually calls the direct sum $\bigoplus_{p,q} {}^pH^{p+q}/{}^{p+1}H^{p+q}$ the associated graded C-module of the filtered graded C-module H, and, further, one denotes $\bigoplus_{p,q} E_r^{p,q} = E_r$ and $\bigoplus_{p,q} E_\infty^{p,q} = E_\infty$. With this, the above lemmas can be put together to form

THEOREM 3. *The Dolbeault groups $H^p(M, \Omega^q)$ form the term E_1 of a spectral sequence, whose term E_∞ is the associated graded C-module of the conveniently filtered de Rham groups. The spectral sequence is stationary after a finite number of steps, and $E_\infty = E_N$ for N sufficiently large.*

5. Theorem 3 states the general relation between the Dolbeault and the de Rham groups. Several corollaries can be obtained. So some considerations about the dimensions of the involved groups easily yield Theorems 1 and 2. In fact, one first observes that it follows from equation (4) that

$$\dim H^n = \sum_{i=0}^{m} \dim \frac{{}^iH^n}{{}^{i+1}H^n}. \tag{4}$$

The left-hand side here is just the nth Betti number b_n; the right-hand side can be written as $\sum_{p+q=n} \dim E_\infty^{p,q}$, because of property b. Thus

$$b_n = \sum_{p+q=n} \dim E_\infty^{p,q}. \tag{5}$$

Since relation (3) implies that $\dim E_{r+1}^{p,q} \leqslant \dim E_r^{p,q}$ and because $E_\infty^{p,q} = E_N^{p,q}$ for N sufficiently large, we have

$$b_n = \sum_{p+q=n} \dim E_N^{p,q} \leqslant \sum_{p+q=n} \dim E_{N-1}^{p,q} \leqslant \ldots \leqslant \sum_{p+q=n} \dim E_1^{p,q}.$$

This proves Theorem 2, as Lemma 1 shows.

The Euler characteristic χ is defined as $\chi = \sum_{r=0}^{2m} (-1)^r b_r$. Thus, by equation (5),

$$\chi = \sum_{p,q=0}^{m} (-1)^{p+q} \dim E_\infty^{p,q}.$$

But, using the fact that E_{r+1}^{p+q} is isomorphic to the "cohomology groups" of $E_r^{p,q}$ by the differential operator d_r, which raises the "total degree" $p+q$ by one (see

property a), one easily verifies that $\sum_{p,q} (-1)^{p+q} \dim E_{r+1}^{p,q} = \sum_{p,q} (-1)^{p+q} \dim E_r^{p,q}$ and hence Theorem 1 follows.

6. Finally, one may remark that for the investigation of complex manifolds not only the Dolbeault groups as the term E_1 of the spectral sequence may be of interest, but also the other terms. The meaning of the terms $E_2^{p,q}$ is very obvious. The differential operator ∂ induces homomorphisms of the factor groups

$$\frac{\{\varphi \mid \bar{\partial}\varphi = 0\}}{\{\varphi \mid \varphi = \bar{\partial}\psi\}},$$

hence of the Dolbeault groups:

$$\ldots \xrightarrow{\partial} H^q(M, \Omega^{p-1}) \xrightarrow{\partial} H^q(M, \Omega^p) \xrightarrow{\partial} H^q(M, \Omega^{p+1}) \xrightarrow{\partial} \ldots . \tag{6}$$

One has $\partial^2 = 0$. The deviation from exactness of this sequence is measured by the groups $E_2^{p,q}$ which are the factor groups of the kernels modulo the images of this sequence. This means, in other words, that

$$E_2^{p,q} \cong \frac{\{\varphi \text{ of type } (p,q) \mid \bar{\partial}\varphi = 0;\ \partial\varphi = \bar{\partial}\alpha\}}{\{\varphi \text{ of type } (p,q) \mid \varphi = \partial\psi + \bar{\partial}\beta;\ \bar{\partial}\psi = 0\}}.$$

The other terms of the spectral sequence can be interpreted analogously.

In the Kähler case, the homorphisms ∂ in formula (6) are all zero. Only the Dolbeault groups are of interest then, since we have $E_1^{p,q} \cong E_2^{p,q} \cong \ldots \cong E_\infty^{p,q}$, and hence one has the well-known result

$$H^n \cong \bigoplus_{p+q=n} H^p(M, \Omega^q),$$

including relations (1) and (2).

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[1] P. Dolbeault, "Sur la cohomologie des variétés analytiques complexes," *Compt. rend. Acad. sci.* (Paris), **236**, 175–177, 1953.

[2] J.-P. Serre, "Un Théorème de dualité," *Com. Math. Helv.*, **29**, 9–26, 1955.

[3] *Séminaire de topologie algébrique de l'École normale superieure*, **1950–1951**, exposés par H. Cartan et J.-P. Serre. The edition by the Massachusetts Institute of Technology has an appendix on spectral sequences.

[4] J. Leray, "L'Anneau spectral et l'anneau filtré d'homologie," *J. Math. Pure. Appl.*, **29**, 1–139, 1950.

THE INTEGRAL OF THE ASSOCIATED LEGENDRE FUNCTION*

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In theoretical physics it is often necessary to expand functions, $\rho(r, \theta, \phi)$, in terms of the surface spherical harmonics, $P_n^m(\cos\theta) \exp(im\phi)$. If the function to be expanded is independent of the angle θ, the evaluation of the expansion coefficients requires a knowledge of the set of integrals

$$R_n^m = \int_{-1}^{1} P_n^m(\mu)\, d\mu \tag{1}$$

for zero or positive integer values of m and n, with $n \geq m$. Here the $P_n^m(\mu)$ are the associated Legendre functions of the first kind:[1]

$$P_n^m(\mu) = \frac{(1-\mu^2)^{m/2}}{2^n n!} \frac{d^{m+n}}{d\mu^{m+n}} (\mu^2 - 1)^n. \tag{2}$$

The expansion of $\exp(im\phi)$ in spherical harmonics is typical of the problems requiring a knowledge of the R_n^m:

$$e^{im\phi} = \sum_{n=|m|}^{\infty} \left(\frac{2n+1}{2}\right) \frac{(n-|m|)!}{(n+|m|)!} R_n^{|m|} P_n^{|m|}(\cos\theta) e^{im\phi}. \tag{3}$$

The integrals R_n^m can easily be obtained by integrating termwise the polynomial form for the associated Legendre functions (see eq. [16]). However, the answers obtained in this manner are in the form of an awkward summation. We have obtained, in a different manner, the following set of comparatively simple results which to the best of our knowledge are not listed in the standard references.

$$R_n^m = \frac{2m}{n} \cdot \frac{[(n/2)!]^2\,(n+m)!}{[(n-m)/2]!\,[(n+m)/2]!\,(n+1)!} \qquad (n \text{ even}, m \text{ even}), \tag{4}$$

$$R_n^m = \frac{m\pi}{n 2^{2n+1}} \cdot \frac{(n+m)!\,(n+1)!}{\{[(n+1)/2]!\}^2\,[(n-m)/2]!\,[(n+m)/2]!} \quad (n \text{ odd}, m \text{ odd}), \tag{5}$$

$$R_n^m = 0 \qquad (m+n \text{ odd}). \tag{6}$$

Equation (6) follows directly from the fact that the integrand is an odd function. The other expressions can be derived from the recursion relation,[2]

$$P_n^m(\mu) = 2(m-1) \frac{\mu}{\sqrt{1-\mu^2}} P_n^{m-1}(\mu) - (n-m+2)(n+m-1) P_n^{m-2}(\mu). \tag{7}$$

From the definition of the associated Legendre functions, equation (2), and integration by parts, it follows that

$$\int_{-1}^{1} \frac{\mu}{\sqrt{1-\mu^2}} P_n^{m-1}(\mu)\, d\mu = \frac{1}{m} \int_{-1}^{1} P_n^m(\mu)\, d\mu. \tag{8}$$

Thus, integrating both sides of equation (7) and simplifying,

$$\int_{-1}^{1} P_n^m(\mu)\, d\mu = \frac{m}{(m-2)}\,(n-m+2)\,(n+m-1)\int_{-1}^{1} P_n^{m-2}(\mu)\, d\mu. \quad (9)$$

Equation (9) then provides a recursion formula for the R_n^m. If m and n are even,

$$R_n^m = \frac{m[(n/2)!]^2\,(n+m)!R_n^2}{2n[(n-m)/2]!\,[(n+m)/2]!\,(n+1)!}. \quad (10)$$

And if both m and n are odd,

$$R_n^m = \frac{2m(m+n)!\,\{[(n+1)/2]!\}^2\,R_n^1}{(n+1)\,(n+1)!\,[(n-m)/2]!\,[(n+m)/2]!}. \quad (11)$$

The value of R_n^2 is found by substituting the definitional relation, equation (2), in equation (1) and integration by parts twice, to obtain

$$R_n^2 = \frac{1}{2^{n-1}n!}\left[\mu\frac{d^n}{d\mu^n}(\mu^2-1)^n\right]_{-1}^{1} - \frac{1}{2^{n-1}n!}\left[\frac{d^{n-1}}{d\mu^{n-1}}(\mu^2-1)^n\right]_{-1}^{1}. \quad (12)$$

These two terms are related to the values of the Legendre functions for $\mu = \pm 1$. The first term is equal to 4, and the second term is zero for all even values of n. Thus $R_n^2 = 4$, and equation (4) follows from equation (10).

The following expression for R_n^1 was obtained by MacRoberts:[3]

$$R_n^1 = \frac{\pi(n+1)}{n2^{2n+2}}\,\frac{[(n+1)!]^2}{\{[(n+1)/2]!\}^4}. \quad (13)$$

Substituting this relation into equation (11) leads to equation (5).

It is interesting to compare the expressions for R_n^m given by equations (4), (5). and (6) with those obtained by a direct integration of the associated Legendre functions. If $n+m$ is even, equation (2) can be rewritten in the form

$$P_n^m(\mu) = \frac{1}{2^n}(1-\mu^2)^{m/2}\sum_{l=0}^{(n-m)/2}(-1)^l\,\frac{(2n-2l)!\,\mu^{n-m-2l}}{(n-m-2l)!!(n-l)!} \quad (14)$$

Now, making use of the beta function relation,

$$\int_{-1}^{1}(1-\mu^2)^{m/2}\mu^{(n-m-2l)}\,d\mu = B\left(\frac{n-m-2l+1}{2},\frac{m+2}{2}\right) = \frac{\Gamma[(n-m-2l+1)/2]\,\Gamma[(m+2)/2]}{\Gamma[(n-2l+3/2]}. \quad (15)$$

Direct substitution of equation (15) into the integral of equation (14) gives

$$R_n^m = \frac{1}{2^n}\sum_{l=0}^{(n-m)/2}\frac{(-1)^l(2n-2l)!\,\Gamma[(n-m-2l+1)2]/\,\Gamma[(m+2)/2]}{(n-m-2l)!!(n-l)!\,\Gamma[(n-2l+3)/2]}. \quad (16)$$

Equation (16) is somewhat more complicated than equations (4) and (5), since it involves the summation over l.

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[1] Some authors, including MacRoberts, Magnus, and Oberhettinger, define Ferrers' associated Legendre function of the first kind as $(1)^m$ times the definition given in eq. (2). Our definition, however, agrees with Jahnke and Emde and with Whittaker and Watson.

[2] H. Margenau and G. M. Murphy, *Mathematics of Physics and Chemistry* (New York: D. Van Nostrand Co., 1943), p. 103, eq. (3-45).

[3] A. Erdelyi, "Higher Transcendental Functions" (Bateman Manuscript Project) (McGraw-Hill, 1953) **1**, 172, eq. (27). See also T. M. MacRoberts, *Quart. J. Math.* (Oxford ser.), **11**, 95 and 96, 1940; T. M. MacRoberts, *Spherical Harmonics* (New York: Dover Publications, Inc., 1947), p. 367, ex. 86(ii), together with p. 143, ex. 10, and p. 125, eq. (14).

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*SIMPLE SUBVARIETIES OF ANALYTIC VARIETIES**

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1. *Introduction.*—Let k be a field. We make the simplifying assumption that its characteristic p is *not* 0. To every prime ideal $\mathfrak{p}$ in the power series ring $A = k[[X_1, \ldots, X_n]]$ we associate an object $V(\mathfrak{p})$ called an *analytic variety over* k.[1] The dimension of the local ring $A/\mathfrak{p}$ is called the *dimension* of $V(\mathfrak{p})$.[2] If $\mathfrak{p} \subset \mathfrak{p}'$, we say that $V(\mathfrak{p}')$ is a *subvariety* of $V(\mathfrak{p})$. A subvariety $V(\mathfrak{p}')$ of an analytic variety $V(\mathfrak{p})$ is called *simple* if its local ring $(A/\mathfrak{p})_{(\mathfrak{p}'/\mathfrak{p})} = A_{\mathfrak{p}'}/\mathfrak{p}A_{\mathfrak{p}'}$ is *regular*.[2] We intend to show that Zariski's generalized Jacobian criterion for simple subvarieties of algebraic varieties may be extended to analytic varieties. The proof closely follows the proof of Zariski's criterion given in a recent book of ours.[3]

2. *Notions about Derivations and Differentials.*—Let A be any commutative ring (not necessarily with unit). One usually considers derivations of A with values in some overring of A. It is convenient to have here the more general notion of a derivation of A taking its values in an *A-module M:* such a derivation is an additive mapping of A into M such that $D(ab) = a \cdot Db + b \cdot Da$. If f is a homomorphism of M into another A-module M', then $f \circ D$ is also a derivation of A.

One can construct an A-module $\mathfrak{D}(A)$ and a derivation $d: A \to \mathfrak{D}(A)$ which are *universal* for the derivations of A, in the sense that, given any derivation $D: A \to M$, there exists a homomorphism $f: \mathfrak{D}(A) \to M$ such that $D = f \circ d$. In fact, one associates, with every element x of A, a symbol m_x, one constructs the free module $F(A) = \sum_{x \in A} A \cdot m_x$, and $\mathfrak{D}(A)$ is then the factor module of $F(A)$ by the submodule generated by all the elements $m_{x+y} - m_x - m_y$ and $m_{xy} - x \cdot m_y - y \cdot m_x$. If we denote by dx the class of m_x in $\mathfrak{D}(A)$, then d is a derivation of A, which obviously enjoys the universal mapping property. We say that $\mathfrak{D}(A)$ is *the module of differentials of* A.

More generally, given another ring B and a homomorphism $h: A \to B$, every B-module M is also an A-module, and we can consider derivations of A into B-modules (here we have $D(aa') = h(a)Da' + h(a')Da$). There still exists a *universal B-module* $\mathfrak{D}(B, A)$ for these derivations: the factor module of the free module $F(B, A) =$

$\sum_{\epsilon A} B \cdot m_x$ by the submodule $R(B, A)$ generated by all the elements $m_{x+y} - m_x - m_y$, $m_{xy} - h(x) \cdot m_y - h(y) \cdot m_x$. We still denote by dx (or $d_{B,\,A}x$, or $d_A x$ to avoid confusion) the class of m_x in $\mathfrak{D}(B, A)$. The B-module $\mathfrak{D}(B, A)$ is isomorphic to the tensor product $B \otimes_A \mathfrak{D}(A)$ (the structure of A-module of B being defined by h). We have $\mathfrak{D}(A) = \mathfrak{D}(A, A)$.

If we have four rings A, A', B, B' with commuting homomorphisms

$$\begin{array}{ccc} A & \xrightarrow{h} & B \\ \downarrow{\scriptstyle u} & & \downarrow{\scriptstyle v}, \\ A' & \xrightarrow{h'} & B' \end{array}$$

the mapping $\sum_i b_i \cdot m_{a_i} \to \sum_i v(b_i) \cdot m_{u(a_i)}$ ($a_i \in A$, $b_i \in B$) of $F(B, A)$ into $F(B', A')$ carries $R(B, A)$ into $R(B', A')$. We thus have a mapping $\mathfrak{D}(B, A) \to \mathfrak{D}(B', A')$, which is a homomorphism for their structures of B-modules (the structure of B-module of $\mathfrak{D}(B', A')$ being defined by v). In particular, a homomorphism of A into A' defines a homomorphism of $\mathfrak{D}(A)$ into $\mathfrak{D}(A')$.

Let $\mathfrak{p}$ be an ideal in A. From the homomorphisms $\mathfrak{p} \to A \to A/\mathfrak{p} \to 0$, we deduce homomorphisms $\mathfrak{D}(A/\mathfrak{p}, \mathfrak{p}) \xrightarrow{s} \mathfrak{D}(A/\mathfrak{p}, A) \xrightarrow{t} \mathfrak{D}(A/\mathfrak{p}, A/\mathfrak{p}) \to 0$ (notice that $\mathfrak{p}$ operates trivially on $A/\mathfrak{p}$).

LEMMA 1. *The preceding sequence is exact.*

Actually, the only point which is not absolutely obvious is the fact that every element ω of the kernel of t is in the image of s. Since $t(\omega) = 0$, we see, by "lifting" ω in the free module $F(A/\mathfrak{p}, A)$, that ω is a linear combination of elements like $da\text{-}da'\text{-}da''$, where $a \equiv a' + a''$ $(\mathfrak{p})$, or $da\text{-}a'da''\text{-}a''da'$, where $a \equiv a'a''$ $(\mathfrak{p})$; as all such elements are of the form dz with z in $\mathfrak{p}$, ω is in the image of s. *QED.*

Simple examples—e.g., the existence of a nontrivial derivation of $2Z$ into $Z/(2)$—show that s need not be a monomorphism. In other words, $\mathfrak{D}$ is a *right exact functor* (in its second variable), but it is *not* left exact. It could be interesting to study its satellites.

If, instead of rings, we consider *algebras* over a fixed ring k, the consideration of the derivations which are k-linear leads, in a similar way, to the definition of *relative* differential modules $\mathfrak{D}_k(B, A)$. Analogous property of right exactness.

3. *Statement of the Theorem and Reduction to a Lemma.*—Every derivation of $A = k[[X_1, \ldots, X_n]]$ is trivial on $A^p = k^p[[X_1{}^p, \ldots, X_n{}^p]]$. If (z_j) denotes a p-basis of k (the finite products $\Pi\, z_j{}^{n(j)}$, where $0 \leq n(j) \leq p - 1$, forming a linear basis of k over k^p), then A is a "topologically" free module over A^p, with the finite products $\prod_{i,j} X_i{}^{n(i)} z_j{}^{n(j)}$ $(0 \leq n(i), n(j) \leq p - 1)$ as basic elements.[4] It follows that every derivation of A (with values in any A-module) is a linear combination of the derivations D_i, D_j' defined by $D_i(X_{i'}) = \delta_{ii'}$, $D_i(z_j) = 0$, $D_j'(X_i) = 0$, $D_j'(z_{j'}) = \delta_{jj'}$ (consequently, every such derivation is *continuous* for the local ring topology of A, a circumstance which does not happen in characteristic 0). In other words, $\mathfrak{D}(A, A)$ is a "topologically" free A-module, freely generated by the elements dX_i, dz_j.[4]

Consider now two prime ideals $\mathfrak{p}$, $\mathfrak{q}$ of A such that $\mathfrak{q} \subset \mathfrak{p}$; denote by d and e the

dimensions of $V(\mathfrak{p})$ and $V(\mathfrak{q})$, and by x_i the $\mathfrak{p}$-residue of X_i. Let (F_α) be any basis of $\mathfrak{q}$.

THEOREM. *The following assertions are equivalent:*

1. *$V(\mathfrak{p})$ is a simple subvariety of $V(\mathfrak{q})$.*
2. *The Jacobian matrix $((D_iF_\alpha)(x), (D_j'F_\alpha)(x))$ has rank $n - e$.*

If the complete local ring $k[[x]] = A/\mathfrak{p}$ is separable over k,[5] then assertion 1 is equivalent to

3. *The matrix $((D_iF_\alpha)(x))$ has rank $n - e$.*

By definition, relation 1 means that $A_\mathfrak{p}/\mathfrak{q}A_\mathfrak{p}$ is a regular local ring (of dimension $e - d$). Since $A_\mathfrak{p}$ is a regular local ring of dimension $n - d$,[6] this means that $\mathfrak{q}A_\mathfrak{p}$ is generated by $n - e$ elements of some regular system of parameters of $A_\mathfrak{p}$.[7] If we set $R = A_\mathfrak{p}$, $\mathfrak{m} = \mathfrak{p}A_\mathfrak{p}$, this is equivalent to saying that, in the $(n - d)$-dimensional vector space $\mathfrak{m}/\mathfrak{m}^2$ over $R/\mathfrak{m}$, the subspace $(R\mathfrak{q} + \mathfrak{m}^2)/\mathfrak{m}^2$ generated by the classes of the F_α's has dimension $n - e$. (Notice that this subspace has, at any rate, a dimension $\leq n - e$, since $R/R\mathfrak{q}$ has dimension $e - d$.)

Now let $(u_1, \ldots, u_{n-d})$ be a regular system of parameters of R. Any derivation D of $\mathfrak{m}$ into $R/\mathfrak{m}$ is obviously trivial on $\mathfrak{m}^2$ and is uniquely determined by the knowledge of the values $D(u_i)$ (which may be arbitrarily selected in $R/\mathfrak{m}$): if $x = \sum_i a_iu_i \in \mathfrak{m}$, then $Dx = \sum_i \bar{a}_iDu_i$ ($\bar{a}_i$ = $\mathfrak{m}$-residue of a_i). Thus $\mathfrak{D}(R/\mathfrak{m}, \mathfrak{m})$ is an $(n - d)$-dimensional vector space over $R/\mathfrak{m}$, generated by the elements $d_\mathfrak{m}u_i$. The formula $d_\mathfrak{m}(\sum_i a_iu_i) = \sum_i \bar{a}_id_\mathfrak{m}u_i$ shows that $\mathfrak{D}(R/\mathfrak{m}, \mathfrak{m})$ is *canonically isomorphic to* $\mathfrak{m}/\mathfrak{m}^2$.

In view of this isomorphism, we have to express the fact that the rank of the system of vectors $(d_\mathfrak{m}F_\alpha)$ in $\mathfrak{D}(R/\mathfrak{m}, \mathfrak{m})$ is $n - e$. We have a homomorphism $\varphi: \mathfrak{D}(R/\mathfrak{m}, \mathfrak{m}) \to \mathfrak{D}(R/\mathfrak{m}, R)$. Since $\mathfrak{D}(A, A)$ is freely generated by the elements dX_i, dz_j, $\mathfrak{D}(A/\mathfrak{p}, A)$ and $\mathfrak{D}(R/\mathfrak{m}, A)$ are also freely generated by these elements. Writing any element u of $R = A_\mathfrak{p}$ under the form $u = a/b$ ($a, b \in A$, $b \notin \mathfrak{p}$), the formula $du = (\bar{b} \cdot da - \bar{a} \cdot db)/\bar{b}^2$ makes sense in $\mathfrak{D}(R/\mathfrak{m}, R)$, since $b^2 \notin \mathfrak{p}$, and shows that $\mathfrak{D}(R/\mathfrak{m}, R)$ is freely generated by the elements dX_i, dz_j. Consequently, the image $\varphi(d_\mathfrak{m}F_\alpha)$ is $\sum_i (D_iF_\alpha)(x)\, dX_i + \sum_j (D_j'F_\alpha)(x)\, dz_j$, and the equivalence of assertions 1 and 2 will be proved if we show that φ is a *monomorphism*. This is obviously equivalent to the following lemma:

LEMMA 2. *Every derivation of $\mathfrak{m}$ with values in $R/\mathfrak{m}$ may be extended to R.*

Similarly, the equivalence of assertions 1 and 2 is reduced, by considering the relative differential modules $\mathfrak{D}_k$, to the following lemma:

LEMMA 3. *If $A/\mathfrak{p} = k[[x]]$ is separable over k, then every k-derivation of $\mathfrak{m}$ into $R/\mathfrak{m}$ may be extended to R.*

4. *Proof of the Lemmas.*—It is sufficient to extend to R the derivations Δ_j of $\mathfrak{m}$ defined by $\Delta_j(u_i) = \delta_{ij}$. By Cohen's theorem,[8] the completion $\hat{R}$ is a power series ring $K[[u_1, \ldots, u_{n-d}]]$ over some representative field K (isomorphic to $R/\mathfrak{m}$), and the derivation $G(u_1, \ldots, u_{n-d}) \to (\partial G/\partial u_j)\ (0, \ldots, 0)$ extends Δ_j to $\hat{R}$. *QED.*

The fact that the representative field K cannot always be chosen so as to contain k shows why the derivations D_j' of k over k^p have to appear in the general case. However, if $A/\mathfrak{p} = k[[x]]$ is separable over k, then a result of Nagata's[9] shows the existence of a system of parameters $(y_1, \ldots, y_d)$ in $A/\mathfrak{p}$ such that $k((x))$ is separably algebraic over $k((y))$. Then representatives $Y_1, \ldots, Y_d$ of $y_1, \ldots, y_d$ in A are ana-

lytically independent over k, and $R = A_\mathfrak{p}$ contains $K' = k((Y_1, \ldots, Y_d))$ as a subfield. Since $R/\mathfrak{m} = \hat{R}/\hat{R}\mathfrak{m}$ is separably algebraic over the image of K', Hensel's lemma proves that there exists in $\hat{R}$ a representative field K containing K' and therefore also k.[10] This proves Lemma 3. An analogous method may be used in characteristic 0.

5. *Other Proof in Case* $[k:k^p]$ *Is Finite.*—For proving that the mapping φ: $\mathfrak{D}(R/\mathfrak{m}, \mathfrak{m}) \to \mathfrak{D}(R/\mathfrak{m}, R)$ is a *monomorphism*, we consider the exact sequence (sec. 2, Lemma 1): (S) $\mathfrak{D}(R/\mathfrak{m}, \mathfrak{m}) \xrightarrow{\varphi} \mathfrak{D}(R/\mathfrak{m}, R) \to \mathfrak{D}(R/\mathfrak{m}, R/\mathfrak{m}) \to 0$. If we set $[k:k^p] = p^s$, we have $[k((X)):k^p((X^p))] = p^{n+b}$, $[k((x)):k^p((x^p))] = p^{d+s}$,[11] and the dimensions of $\mathfrak{D}(R/\mathfrak{m}, R)$ and $D(R/\mathfrak{m}, R/\mathfrak{m})$ are $n + s$ and $d + s$. Since the dimension of $\mathfrak{D}(R/\mathfrak{m}, \mathfrak{m})$ has been proved to be equal to $n - d = (n + s) - (d + s)$ (cf. sec. 3), the exactness of (S) shows that φ is a monomorphism. A similar proof, using the modules $\mathfrak{D}_k$, gives the equivalence of assertions 1 and 3 in the separable case.

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[1] C. Chevalley, "Intersections of Algebraic and Algebroid Varieties," *Trans. Am. Math. Soc.*, **57**, 1–85, 1945. Chevalley restricts himself to the case where k is algebraically closed.

[2] For the general notions about local rings see P. Samuel, *Algèbre locale* ("Mem. Sci. Math.," No. 123 [Paris, 1953]).

[3] P. Samuel, "Méthodes d'algèbre abstraite en géométrie algébrique," *Ergeb. Math.*, N.F., Heft 4, 1955. See chap. ii, sec. 4.

[4] If $[k:k^p]$ is infinite, the module $\mathfrak{D}(A, A)$ must be considered as a subset of the infinite product $E = \prod_i Adx_i \times \prod_j Adz_j$. Then the infinite sum notation $\sum_i a_i dX_i + \sum_j b_j dz_j$ for an element of $\mathfrak{D}(A, A)$ is justified if one considers E as a topological module, either with the topology defined by the submodules $\mathfrak{X}^n \cdot E$ (where $\mathfrak{X}$ denotes the ideal of A generated by $X_1, \ldots, X_n$) or with the product topology of discrete topologies. Similarly for $\mathfrak{D}(A/\mathfrak{m}, A)$. For $\mathfrak{D}(R/\mathfrak{m}, A)$ and $\mathfrak{D}(R/\mathfrak{m}, R)$ one can only use the product topology of discrete topologies.

[5] According to Nagata ("Note on Complete Local Integrity Domains," *Mem. Coll. Sci. Univ. Kyoto*, ser. A, No. 3, pp. 271–278, 1953), this means that the completed tensor product $k[[x]] \otimes_k k^{p^{-1}}$ is an integral domain. This implies that the field $k((x))$ is separable over k and is equivalent to that property if $[k:k^p]$ is finite.

[6] Samuel, *Algèbre locale*, Prop. 1, p. 52.

[7] *Ibid.*, p. 29.

[8] *Ibid.*, Theorème 2, p. 48.

[9] Nagata, *op. cit.*, Theorem 3.

[10] Proof as in Samuel, *Algèbre locale*, corollary, p. 45.

[11] Proof as in Samuel, "Méthodes d'algèbre abstraite en géométrie algébrique," chap. ii, sec. 4, No. 2, Lemme 1.

ON THE PROPERTIES OF CERTAIN TRINOMIAL EQUATIONS IN A FINITE FIELD

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In a recent paper[1] Mrs. E. H. Pearson and the writer considered certain types of trinomial congruences, and in later papers the writer[2, 3] developed this topic further and pointed out[2] a relation of such questions to Case II of Fermat's Last Theorem. In the present paper we shall continue this investigation.

Let p be an odd prime and $F[p^n]$ denote a finite field of order p^n, and consider the solutions s, t of

$$g^{i+cs} + g^{j+mt} = 1, \tag{1}$$

where g is a multiplicative generator of the nonzero elements of $F[p^n]$; i is a fixed integer in the set $0, 1, \ldots, c-1$; j is a fixed integer in the set $0, 1, \ldots, m-1$; $p^n - 1 \equiv 0 \pmod{m}$, $p^n - 1 \equiv 0 \pmod{c}$; s is in the range $0, 1, \ldots, m-1$; and t is the range $0, 1, \ldots, c-1$. Let the number of sets of solutions s, t of the above type be $(i, j)_{cm}$. Then (noting relation [2] of the present paper) the maximum value for $(i, j)_{cm}$ is m if $c \geq m$. We now define the particular types of equations we mainly treated in our previous papers. If there exists a j for a fixed i such that $(i, j)_{cm} = m$, with $cm = p^n - 1$, we shall call this "Case A" of equation (1), and if i is fixed and $(i, j)_{cm} = 1$ for each j in the set $0, 1, \ldots, m-1$, we shall call this "Case B" of equation (1).

In our previous paper[3] we obtained some relations involving the (i, j)'s which follow from the assumptions of Case A; but in all of them we assume that $c \equiv 0 \pmod{m}$ in the relation $p^n - 1 = cm$. We shall now consider the case where c is prime to m. The relations obtained here will be somewhat different from those obtained in our previous paper. We shall again need the two following relations.

Now consider relation (1) with the number m an integer >2. We note that

$$\sum_{j=0}^{m-1} (i, j)_{cm} = m - \begin{cases} 0 & \text{if } i \neq 0, \\ 1 & \text{if } i = 0. \end{cases} \tag{2}$$

Also, we shall set $(i, j)_{cc} = (i, j)_c$. We then have[4]

$$\sum_{i=0}^{c-1} (b, i)_c (b - a, i - d)_c + A_1 = \sum_{i=0}^{c-1} (d, i)_c (d - a, i - b)_c + A_2, \tag{3}$$

where $A_1 = 0$ except for $a \equiv b \equiv 0 \pmod{c}$ when it is m, and $A_2 = 0$ except when $a \equiv 0, d \equiv 0 \pmod{c}$ when $A_2 = m$.

Set $p^n - 1 = v$. Assume $b \not\equiv 0 \pmod{v}$, $d \not\equiv 0 \pmod{v}$. Then equation (3) becomes, since $A_1 = A_2 = 0$ because of the restrictions just mentioned,

$$\sum_{i=0}^{v-1} (b, i)_v (b - a, i - d)_v = \sum_{i=0}^{v-1} (d, i)_v (d - a, i - b)_v. \tag{4}$$

Now assume that the relations $d \equiv 0 \pmod{m}$ and $a \equiv 0 \pmod{c}$ do not both hold, and then set $a = a_1 + c, \ldots, a_1 + (m-1)c$, in turn, throughout and add; then, using the following (relation [13], p. 1283 of Paper T),

$$\sum_{s=0}^{m-1} (k - a_1 + sm, i - d)_v = (k - a_1, i - d)_{cv}, \tag{5}$$

for any k, we find

$$\sum_{i=0}^{v-1} (b, i)_v (b - a_1, i - d)_{cv} = \sum_{i=0}^{v-1} (d, i)_v (d - a_1, i - b)_{cv}. \tag{6}$$

Set $b = b_1, b_1 + c, \ldots, b_1 + (m-1)c$ in turn and add; we have, if $b_1 \not\equiv 0 \pmod{c}$, using equation (5),

$$\sum_{i=0}^{v-1} (b_1, i)_{cv} (b_1 - a_1, i - d)_{cv} = \sum_{i=0}^{v-1} (d, i)_v (d - a_1, i - b_1)_c. \tag{7}$$

Substitute $i = j, j + c, \ldots, j + (m-1)c$ on the right, and carry out the summation for each j in the set $0, 1, \ldots, c-1$. We find, again using equation (5),

$$\sum_{i=0}^{v-1} (b_1, i)_{cv} (b_1 - a_1, i - d)_{cv} = \sum_{j=0}^{c-1} (d, j)_{vc} (d - a_1, j - b_1)_c. \tag{8}$$

If $d_1 \not\equiv 0 \pmod{m}$, then set, in turn, $d = d_1, d_1 + m, \ldots, d_1 + (c-1)m$ and add; we have, in view of the assumptions stated just below equation (4),

$$\sum_{i=0}^{v-1} (b_1, i)_{cv} (b_1 - a_1, i - d_1)_{cm} = \sum_{\substack{j=0 \\ d_1}}^{c-1} (d_1, j)_{vc} (d_1 - a_1, j - b_1)_c. \tag{9}$$

Set, on the left, $i = i_1, i_1 + m, \ldots, i_1 + (c-1)m$, and collect terms for each i_1 in the set $0, 1, \ldots, m-1$; we find

THEOREM I. *We have, if $p^n - 1 = cm$, p an odd prime, $(c, m) = 1$,*

$$\sum_{i=0}^{m-1} (b, i)_{cm} (b - a, i - d)_{cm} = \sum_{\substack{j=0 \\ d}}^{c-1} (d, j)_{vc} (d - a, j - b)_c, \tag{10}$$

where d ranges over $d, d + m, \ldots, d + (c-1)m$ and where b is any integer such that $b \not\equiv 0 \pmod{c}$, and where a and d are any integers such that we do not have both of the relations $d \equiv 0 \pmod{m}$ and $a \equiv 0 \pmod{c}$ holding.

We shall now consider the applications of equation (10) to the Case A of equation (1) referred to earlier. Assume that $(b, h) = m$, with $(b, i) = 0$, $i \neq h$. The left-hand member of equation (10) reduces, if we assume $a = 0$, to

$$\sum_{h=0}^{m-1} (b, h)_{cm} (b, h - d)_{cm}.$$

For $d \not\equiv 0 \pmod{m}$ the second factor in each term is zero; thus the entire left-hand member of equation (10) is zero. Hence each term in the right-hand member of equation (10) is zero or

$$(d, i)_{vc} (d, i - b)_c = 0, \tag{11}$$

where d is any integer $d \pmod{m}$ in the range $0, 1, \ldots, v-1$, with $b \not\equiv 0 \pmod{c}$, $d \not\equiv 0 \pmod{m}$.

We may then state

THEOREM II. *Let p be an odd prime and $p^n = 1 + cm$, $(c, m) = 1$. Designate*

by $(i, j)_{cm}$ *the number of sets of solutions* s, t $(0 \leq s < m, 0 \leq t < c)$ *of the equation*

$$g^{i+cs} + g^{j+mt} = 1,$$

with $(i, j)_c = (i, j)_{cc}$ (i *and* j *fixed*). *Also,* g *is a multiplicative generator of the cyclic group formed by the nonzero elements of the finite field* $F[p^n]$ *of order* p^n. *If there is some* h *such that* $(b, h)_{cm} = m$, *then equation* (11) *holds.*

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[1] E. H. Pearson and H. S. Vandiver, "On a New Problem Concerning Trinomial Congruences Involving Rational Integers," these PROCEEDINGS, **39**, 1278–1285, 1953. This article will be referred to as "Paper T."

[2] H. S. Vandiver, "New Types of Trinomial Congruence Criteria Applying to Fermat's Last Theorem," these PROCEEDINGS, **40**, 248–252, 1954.

[3] H. S. Vandiver, "On Trinomial Equations in a Finite Field," *ibid.*, pp. 1008–1010.

[4] H. S. Vandiver, "On Cyclotomy and Extensions of Gaussian Type Quadratic Relations Involving Numbers of Solutions of Conditional Equations in Finite Fields," *ibid.*, **38**, 984, relation (19), 1952.

REDISTRIBUTION OF CLASSICAL AND QUANTUM DENSITIES

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In many problems the Jeffries-Wentzel-Kramers-Brillouin (JWKB) method forms a convenient bridge between quantum mechanical and classical treatments. The method is often employed as a means of obtaining an approximation to the wave function and is then primarily of use in studying the region where the wave function is sufficiently well approximated by the JWKB formula. A different type of application is found in estimating correction terms to the quantum numbers of the Bohr-Sommerfeld theory, resulting in the employment of half-integral quantum numbers in some cases. The goodness of the approximation through the whole region is not essential in the application just quoted. The present note is concerned with a somewhat similar property, in that the validity of the JWKB approximation throughout the whole region for which conclusions are drawn is not essential.

The relation in its simplest form states that, if particles impinge from right to left on a completely reflecting one-dimensional potential barrier, the integral of the quantum mechanical density taken from the barrier interior to one of the nodes of the wave function on the right of the barrier is equal to the integral of the classical particle density from the classical turning point to the same node of the wave function, provided that the JWKB approximation applies at the node. A similar statement can be made about radial wave functions of three-dimensional problems. However, in this case the quantum mechanical angular momentum $L\hbar$ is replaced in the classical comparison problem by $[L(L+1)]^{1/2}\hbar$, and the integration in the barrier interior is made from $r = 0$ as the lower limit.

This relation is responsible to a considerable degree for the rather high accuracy in the representation of Coulomb Excitation[1] by the semiclassical treatment[2] which pictures the approach of the particle by classical dynamics employing quantum mechanics for the remainder of the process.

The proof will be presented for the case of radial motion of two colliding particles. The one-dimensional problem mentioned first is covered by this proof almost without change. The following notation will be used:

r = Distance between the particles
R = Classical distance of closest approach
$u(r)$ = $(2\mu/\hbar^2)U(r)$
$U(r)$ = Potential energy, including the centrifugal barrier
$V(r)$ = Potential energy
$v(r)$ = Classical velocity at distance r
v_∞ = Classical velocity for $r = \infty$
E = Energy of relative motion
$F(r)$ = r times the radial wave function
k^2 = $(2\mu/\hbar^2)E$
p_φ = angular momentum in classical theory

The radial equation is

$$\frac{d^2F}{dr^2} + [k^2 - u(r)]F = 0. \tag{1}$$

From two such equations, at two energies but with the same $u(r)$ which is assumed to be independent of k, there follows the Green's theorem relation

$$\int_0^r F(r, k_2)F(r, k_1)\, dr = \frac{F(r, k_2)\partial F(r, k_1)/\partial r - F(r, k_1)\partial F(r, k_2)/\partial r}{k_2^2 - k_1^2}. \tag{2}$$

At a node of $F(r, k_1) = 0$ the numerator on the right side consists of one term. Making k_2 approach k_1, the contribution of this term to the right side approaches the product of $(\partial F/\partial r)^2$ at the node by the sensitivity of the value of r at the node to k^2. If $\hbar$ is decreased, the wave length is shortened, and for the same amplitude $|\partial F/\partial r|$ increases. The sensitivity of the node position to k^2 can be expected to decrease because k^2 is larger for smaller values of $\hbar$. A simple consideration shows that in the limit $k_1 = k_2$ the product just mentioned is independent of $\hbar$, provided that the JWKB approximation is used for the representation of F. Since the JWKB approximation is the geometrical optics approximation to the wave function, it is seen that in the limit of $\hbar = 0$ the integral on the left side of equation (2) approaches the classical density. The correctness of the theorem thus depends only on the disappearance of $\hbar$ from the right side of equation (2) at the node of F. Since the wave length is proportional to $\hbar$, the value of $\partial F/\partial r$ at the node must be proportional to $1/\hbar$; $\partial r_n/\partial(k^2)$, the sensitivity of the node position to k^2, is, on the other hand, proportional to $\hbar^2$; the product of the two factors on the right side is therefore independent of $\hbar$, and the agreement of classical and quantum calculations follows.

The reasoning cannot be applied to the JWKB approximation itself because the differential equation satisfied by this approximation contains k^2 not only in the

additive form as in equation (1) but also in the denominator of correction terms to the potential energy. There is thus no direct implication regarding the density integral of the JWKB approximation. On the other hand, for $h \to 0$ the whole region to the right of the classical turning point is represented nicely by the JWKB approximation, so that the evaluation of the density integral can be made in this limit by means of classical mechanics. Also in the limit $h \to 0$ the extra terms in the differential equation satisfied by the JWKB approximation are negligible.

Even though the proof has been seen not to require explicit calculation but to depend only on general properties of the JWKB approximation, it appears desirable to consider the quantities more explicitly. The equation expressing the fact that $r = r_n$ is a node of $F(r, k_1)$ is

$$F(r_n(k_1), k_1) = 0, \tag{3}$$

where the functional dependence of r_n on k is explicitly considered. It follows from equation (3) that to within the first order of small quantities

$$F(r_n(k_1), k_2) = [r_n(k_1) - r_n(k_2)] \left(\frac{\partial F}{\partial r}\right)_n, \tag{3.1}$$

where the subscript n on the right indicates that $r = r_n(k)$ is used. The validity of equation (3.1) is seen by noting that (3) holds also if k_1 is changed in it to k_2. The value of r in equation (3.1) is meant to be $r_n(k_1)$. From equations (2) and (3.1) it follows that

$$\int_0^r F^2 \, dr_n = -\frac{dr_n}{d(k^2)} \left(\frac{\partial F}{\partial r}\right)_n^2. \tag{3.2}$$

The JWKB approximation is

$$F \cong \frac{k^{1/2} \sin \varphi}{[k^2 - u(r)]^{1/4}}, \tag{4}$$

$$\varphi = \int_R^r [k^2 - u(r)]^{1/2} \, dr + \epsilon, \tag{4.1}$$

which is used here without restriction on the value of ϵ but with the assumption

$$\frac{\partial \epsilon}{\partial k} = 0. \tag{4.2}$$

The normalization of the JWKB approximation is such as to make the approximate F asymptotic to a sine function of unit amplitude at $r = \infty$ in agreement with the normalization of the exact F. The condition for having a node is

$$\varphi(r_n) = \pi n. \tag{4.3}$$

It follows from equation (4.3) by differentiation with respect to k^2 that

$$\frac{\partial r_n}{\partial (k^2)} = -\frac{\hbar^2}{4\mu} [E - U(r)]^{-1/2} \left[\int_R^r [E - U(r)]^{-1/2} \, dr\right]_n, \tag{4.4}$$

the evaluation of the integral at $r = r_n$ being again indicated by the subscript n. From relation (4) it follows that

$$\left(\frac{\partial F}{\partial r}\right)_n = (-)^n \left[\frac{(2\mu)^{1/2}}{\hbar}\right] E^{1/4} [E - U(r)]^{1/4}. \tag{4.5}$$

The occurrence of $\hbar$ in equations (4.4) and (4.5) is the same as arrived at without the calculation of remaining factors and used in the first proof. Substitution in equation (3.2) by means of equations (4.4) and (4.5) gives

$$\left(\int_0^r F^2\, dr\right)_n \cong \frac{1}{2}\left(\int_R^r \left[1 - \frac{U}{E}\right]^{-1/2} dr\right)_n. \tag{5}$$

If at $r = r_n$ the JWKB formulas are exact, then the sign of approximate equality becomes replaced by an equality sign in formula (5).

In the classical approximation one has

$$\int_0^r F^2\, dr \to \int_R^r \frac{1}{2}\left(\frac{v_\infty}{v}\right) dr = \frac{1}{2}\int_R^r \left[1 - \frac{U}{E}\right]^{-1/2} dr, \tag{6}$$

and the relation is verified. Both proofs involve a convention regarding the correspondence of values of angular momenta in the two calculations. The necessity of this convention may be seen as follows. The relation between the effective potential energy U and the ordinary one is

$$U = V + \frac{L(L+1)\hbar^2}{2\mu r^2}. \tag{7}$$

The classical angular momentum p_φ enters in calculations of radial motion in the combination

$$\frac{V + p_\varphi^2}{2\mu r^2}. \tag{7.1}$$

Since the identification of the right sides of equations (5) and (6) requires that U be the same, it becomes necessary to set

$$p_\varphi^2 = L(L+1)\hbar^2,$$

or, introducing L_c, a classical theory L, by means of

$$\frac{p_\varphi^2}{\hbar^2} = L_c^2$$

one has

$$L_c^2 = L(L+1). \tag{7.2}$$

The argument as presented applies directly also in the case of one-dimensional problems, provided that $U(r)$ is interpreted as the potential energy $V(r)$ and the lower limit of integration is changed from $r = 0$ to $x = -\infty$ in the quantum case.

The theorem as stated can be useful in cases in which there is available a region of good applicability of the JWKB method which is not too far removed from the classical turning point. To obtain such a condition, one needs to have a relatively rapid transition from the gently varying part of the barrier at large r to the region at the classical turning point R in the immediate neighborhood of which the JWKB

approximation does not hold. The usefulness of the theorem lies in the fact that it shows the number of particles to the left of r_n to be the same classically and quantum mechanically. It shows, therefore, that the quantum mechanical gain in numbers of particles to the left of R caused by leakage into the region of negative kinetic energy is compensated for by a decrease on the right of the turning point, which is the region in which the classical density is highest.

The agreement between the classical and quantum quantities can be generalized to the consideration of

$$(\int_0^r F(r, k_1)F(r, k_2)\, dr)_n$$

for the case of $k_1 \neq k_2$. Employing the notation of the phase amplitude method,

$$F = A \sin \varphi; \qquad \frac{d\varphi}{dr} = \frac{k}{A^2}, \tag{8}$$

one has without approximations

$$\int_0^r F_1F_2\, dr = \left\{\frac{1}{2}\left(k_1\frac{A_2}{A_1} + k_2\frac{A_1}{A_2}\right)\sin(\varphi_2 - \varphi_1)\right.$$
$$+ \frac{1}{2}\left(k_1\frac{A_2}{A_1} - k_2\frac{A_1}{A_2}\right)\sin(\varphi_2 + \varphi_1)$$
$$\left. + \left(A_2\frac{dA_1}{dr} - A_1\frac{dA_2}{dr}\right)\sin\varphi_1\sin\varphi_2\right\Big/(k_2^2 - k_1^2), \tag{9}$$

the $d\varphi/dr$ having been eliminated by means of equations (8) and the abbreviated notation $F(r, k_i) = F_i$ being used. If at r the JWKB approximation applies, one has

$$\int_0^r F_1F_2\, dr = \frac{(k_1k_2)^{1/2}}{g_1^{1/4}g_2^{1/4}}\left\{\frac{1}{2}(g_1^{1/2} + g_2^{1/2})\frac{\sin(\varphi_2 - \varphi_1)}{k_2^2 - k_1^2}\right.$$
$$\left. + \frac{1}{2}\frac{g_1^{1/2} - g_2^{1/2}}{k_2^2 - k_1^2}\sin(\varphi_2 + \varphi_1) + \frac{du}{4dr}\frac{\sin\varphi_1\sin\varphi_2}{g_1g_2}\right\}, \tag{10}$$

with

$$g_i = k_i^2 - u(r) \qquad (i = 1, 2) \tag{10.1}$$

and the phases φ_i being defined as in equation (4.1). For small energy differences between the states 1 and 2 and for values of r that are not too large the phase difference $\varphi_2 - \varphi_1$ is small, but the presence of $k_2^2 - k_1^2$ in the denominator of the first term in the braces of equation (10) makes this term finite. In the limit $k_2^2 = k_1^2$ the contribution of this term approaches in this case the expression on the right side of equation (6). If the nodes of F_1 and F_2 are close to r, the two remaining terms in the curly brackets of equation (10) become small in comparison with the first. In fact, if both φ_1 and φ_2 are close to πn, then the last term contains an uncompensated factor $(\varphi_2 - \varphi_1)^2$, which is small in virtue of the smallness of $\varphi_2 - \varphi_1$. The last term contains in addition the factor du/dr, which is usually relatively small in the region in which the JWKB approximation is good. The last term is there-

fore also small under the assumed conditions. The term before last contains the quotient $(g_1^{1/2} - g_2^{1/2})/(k_2^2 - k_1^2)$, which approaches a finite limit as $k_1 \to k_2$. The factor sin $(\varphi_2 + \varphi_1)$, however, vanishes in this limit under the assumed condition of proximity to a node. If the node is exactly between φ_1 and φ_2 on the scale of φ, the term is zero, and it is of the order $\varphi_2 - \varphi_1$ if both F_1 and F_2 have their nodes close to r. It is thus seen that in the limit of equal energies the first term in the braces of equation (10) must reproduce equation (6). For nonvanishing $k_2^2 - k_1^2$ this term may be expressed by means of the following approximate representation of the phase difference:

$$\varphi_2 - \varphi_1 \cong \frac{k_2}{v_{2\infty}} \int_R^r v_2\, dr - \frac{k_1}{v_{1\infty}} \int_R^r v_1\, dr = \frac{\mu}{\hbar} \int_R^r (v_2 - v_1)\, dr \cong$$

$$\frac{\mu}{\hbar} \int_R^r \frac{v_2^2 - v_1^2}{2dr/dt}\, dr = -\frac{(\Delta E)t}{\hbar}. \quad (10.2)$$

The quantities v_1 and v_2 are the velocities which the particle has at r in a classical mechanics calculation. The JWKB approximation is assumed in the first step. While this assumption implies an approximation, the accuracy of the result is somewhat better than that of the JWKB approximation for the wave function. The latter becomes poor at the classical turning point, giving an infinite value for F. The value of $\varphi_2 - \varphi_1$ as obtained from formula (10.2) shows, on the other hand, no such anomaly. The change of the position of the turning point with energy is neglected, since the velocities v_1 and v_2 are both small at the turning point. In the next to the last step in equation (10.2) the quantity dr/dt is introduced. It is meant to be a mean velocity taken between v_1 and v_2. It is seen from equation (10) that in the limit $k_2 \to k_1$ the second term in braces becomes negligible compared with the first not only when $\varphi_1 + \varphi_2$ is close to an even multiple of π but also when it is close to an odd one. In this case φ_1 and φ_2 are odd multiples of $\pi/2$. The last term in braces frequently becomes small in the region of validity of the JWKB method on account of a sufficiently small $d(u/k^2)/d(kr)$, and under these conditions there are therefore points located approximately halfway between the nodes at which the integrals of classical and quantum densities are equal. These points are approximately at the nodes of F, provided that the last term in braces is sufficiently small, as is the case, for example, in the Coulomb Excitation problem. In terms of the approximation of equation (10.2), substitution into equation (10) gives

$$\int_0^r F(r, k_1)F(r, k_2)\, dr \cong \frac{v_\infty}{2(\Delta E/\hbar)} \sin\left[\frac{(\Delta E)t}{\hbar}\right], \quad (10.3)$$

the effect of the last two terms in equation (10) being here neglected. This quantity is related to a similar quantity occurring in the calculation of an excitation process on a semiclassical basis; this may be seen as follows.

A nucleus in an energy state E_0 is being excited to an energy state E_n by a particle incident with velocity v_∞. Particles of fixed angular momentum are considered, and the interaction energy between nucleus and particle will be taken to be a function only of r and of nuclear co-ordinates. Treating the motion of the incident particle classically and the behavior of the nucleus quantum mechanically, the probability of the excitation having taken place as a result of a collision is

$$P_{cl} = \frac{1}{\hbar^2}\left|\int_{-\infty}^{+\infty} H_{no}(r(t))e^{i(\Delta E)t/\hbar}\,dt\right|^2, \tag{11}$$

where

$$\Delta E = E_n - E_0 \tag{11.1}$$

and $H_{no}(r(t))$ is the value of the matrix element of the interaction energy for the value of r corresponding to the time t. The integration over internal nuclear coordinates is supposed to have been performed in obtaining H_{no}. On the other hand, the calculation of the same problem made with neglect of the action of the nucleus on the radial functions $F(r, k)$ and to the first order of the interaction energy gives the same excitation probability as

$$P_{qu} = \frac{16}{\hbar^2 v_\infty{}^f v_\infty{}^i}\left|\int_0^\infty F_1F_2H_{no}(r)\,dr\right|^2, \tag{12}$$

where the superscripts i and f distinguish, respectively, between initial and final values of the velocity. The classical probability can be transformed by introducing the integral of the exponential factor and integrating by parts, thus giving

$$P_{cl} = \frac{1}{\hbar^2}\left|2\int_R^\infty \frac{\sin\left[(\Delta E)t/\hbar\right]}{\Delta E/\hbar} H'_{no}(r(t))\,dr\right|^2, \tag{11'}$$

where the derivative of H'_{no} is taken with respect to r. The quantum expression can be similarly transformed introducing the integral in equation (10.3), giving

$$P_{qu} = \frac{16}{\hbar^2 v_\infty{}^f v_\infty{}^i}\left|\int_0^\infty \frac{F_2\,dF_1/dr - F_1\,dF_2/dr}{k_2{}^2 - k_1{}^2} H'_{no}(r)\,dr\right|^2. \tag{12'}$$

The integral in the last expression contains a factor multiplying H'_{no} which is the integral of the cross-product density and vanishes at $r = 0$. If $H'_{no}(r)$ does not increase too rapidly at $r = 0$, and if F_1 and F_2 are small at $r < R$, one may approximate the factor by means of equation (10.3), making use of equation (2). Neglecting the difference between $v_\infty{}^f$ and $v_\infty{}^i$ outside the integral in equation (12'), the right side of (12') becomes identical with the right side of (11'). There results thus an agreement between the semiclassical and the quantum calculations which is appreciably better than that which can be foreseen on the JWKB approximation as applied directly to the evaluation of equation (12). The latter ceases being valid for values of r somewhat greater than that for the classical turning point R, while the use of equation (10) subordinates the importance of the values of F_1 and F_2 in this region.

In the case of Coulomb Excitation through the action of the nuclear quadrupole moment the matrix element

$$H_{no}(r) = \frac{\text{const.}}{r^3},$$

and there enters therefore a factor $1/r^4$ under the integral in equation (12). While the factor varies rapidly in the region of small r, this variation is not sufficient to offset the importance of the rapid increase of $\int_0^r F_1F_2\,dr$ with increasing r. The

relatively large distance of R from $r = 0$ in comparison with the wave length decreases the importance of variations of r within a wave length. It is thus seen that the good agreement of quantum and semiclassical calculations of Coulomb Excitation can be explained even though the JWKB method is not a good approximation in the region which matters most for the calculation of integrals involving $1/r^3$. The redistribution of the classical density makes the indefinite density integral entering the quantum calculation oscillate about the integral of the corresponding classical quantity. This circumstance is connected with the close equality of quantum mechanical and semiclassical values for the radial integrals entering the Coulomb Excitation formulas.

Although frequent references are made above to Coulomb Excitation, the relation can be applied to other phenomena in quantum mechanics as well as in wave problems in general. Specializing to the case of equal energies, equation (6) gives the transition from wave optics to geometrical optics. The wave equation is then written in the form of equation (1), with E defined as the value of the coefficient $E - U$ at $r = \infty$, and equation (6) is used.

Summary.—It is shown that, if particles impinge from right to left on a completely reflecting one-dimensional potential barrier, the integral of the quantum mechanical density taken from the barrier interior to one of the nodes of the wave function on the right of the barrier is equal to the integral of the classical particle density from the classical turning point to the same node of the wave function, provided that the JWKB approximation applies at the node. Similarly, in the case of radial wave functions the integrals of particle densities computed classically and quantum mechanically from the origin of co-ordinates to a node of the radial wave function are equal provided that the JWKB approximation applies at the node. An extension of the relation to the case of two different quantum states is given. The bearing of the theorem on the success of the semiclassical treatment of Coulomb Excitation is brought out, and the possibility of applications to problems in wave equations of classical physics is pointed out.

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[1] G. Breit and P. B. Daitch, *Phys. Rev.*, **96**, 1447, 1954; Daitch, Lazarus, Hull, Benedict, and Breit, *Phys. Rev.*, **96**, 1449, 1954; L. C. Biedenharn and C. M. Class, *Phys. Rev.*, **98**, 691, 1955; L. C. Biedenharn, J. L. McHale, and R. M. Thaler (to be published).

[2] K. A. Ter Martirosyan, *J. Exptl. Theoret. Phys.* (U.S.S.R.), **22**, 284, 1952; K. Alder and A. Winther, *Phys. Rev.*, **91**, 1578, 1953.

SOLUTION OF THE BOLTZMANN-HILBERT INTEGRAL EQUATION

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1. *Introduction.*—This investigation deals with the classical problem of determining the transport coefficients of viscosity, heat conduction, and diffusion in a model gas consisting of rigid spheres. Boltzmann showed[1] that the solution of this problem requires the determination of the velocity distribution function $f(\mathbf{c}, \mathbf{r}, t)$ in space $\mathbf{r}$ and in velocity-space $\mathbf{c}$. This function is controlled by the Boltzmann[1, 2, 3] integral equation

$$\mathfrak{O}f = \frac{\partial f}{\partial t} + \mathbf{c}\cdot\frac{\partial f}{\partial \mathbf{r}} + \mathbf{F}\cdot\frac{\partial f}{\partial \mathbf{c}} = \frac{\sigma^2}{2}\iint (f'f_1' - ff_1)\,|\mathbf{g}\cdot\mathbf{k}|\,d\mathbf{k}d\mathbf{c}_1, \tag{1}$$

where $\mathbf{F}$ denotes the force acting on the molecules, σ the diameter of the molecules, $\mathbf{k}$ a unit vector in the direction of the line of centers at the time of the encounter, and the prime specifies those molecules which, after encounter, are brought into the element $d\mathbf{c}$ of velocity-space. The search for the solution of this integral equation for the purpose of computing the transport coefficients formed the principal theme of Boltzmann's life's work. These efforts, however, proved fruitless because of the mathematical difficulties involved, and Boltzmann did not succeed in evaluating a single transport coefficient. This is all the more unfortunate, since, as will be shown in the sequel, Boltzmann was actually very close to achieving a solution.

In 1912 the problem was taken up by Hilbert in the last chapter of his book on integral equations.[2] He states that, in contrast to all the previous applications of the theory of integral equations which he made in his book, equation (1) is an intrinsic integral equation and does not have its origin in a differential equation. Hilbert showed that it is possible to carry through the integration over $\mathbf{k}$ in equation (1), thereby achieving a considerable simplification of this integral equation. Putting

$$f^0 = n\left(\frac{m}{2\pi kT}\right)^{3/2}\exp\left(\frac{-mC^2}{2kT}\right), \qquad \mathbf{C} = \mathbf{c} - \mathbf{c}_0,$$
$$f = f^0(1 + \varphi), \qquad \mathbf{p} = \mathbf{C}\sqrt{\frac{m}{2kT}}, \tag{2}$$

where φ is small and n denotes the number density, m the molecular mass, T the temperature, he proves that equation (1) can be transformed into

$$\mathfrak{O}f^0 = -\frac{\sigma^2 n^2 m}{2\pi kT}\left\{M(p)e^{-2p^2}\,\varphi + \frac{1}{\pi}\,e^{-p^2}\int \varphi_1 e^{-p_1^2}\left(R - \frac{2}{R}e^{\omega^2}\right)d\mathbf{p}_1\right\}, \tag{3}$$

where

$$M(p) = 1 + \left(2p + \frac{1}{p}\right)P(p), \qquad P(p) = e^{p^2}\int_0^p e^{-x^2}\,dx, \tag{4}$$

$$R = |\mathbf{p} - \mathbf{p}_1|, \qquad \omega = \frac{pp_1 \sin\theta'}{R}, \tag{5}$$

and θ' denotes the angle between $\mathbf{p}$ and $\mathbf{p}_1$.

Except for an investigation by Boguslawski[4] of the problem of longitudinal oscillations of a gas, and the treatment of the problem of self-diffusion by Pidduck,[5] no attempt has been made to apply Hilbert's integral equation (3) to the determination of the transport coefficients. The problem was finally solved by Chapman[6] and Enskog,[7] who resorted essentially to variational methods. These methods they used effectively also for other gas models.

2. *Method of Solution.*—In evaluating the transport coefficients, one finds that in each case $\mathfrak{I}f^0$ involves only a single spherical harmonic in $\mathbf{p}$. Thus, as will be shown in a subsequent publication, the coefficient of thermal conduction λ is given by

$$\lambda = \frac{4k}{3\pi\sigma^2}\sqrt{\frac{2kT}{m}}\int_0^\infty e^{-p^2}\, a(p)p^6\, dp, \tag{6}$$

where $a(p)$ is a solution of the integral equation

$$M(p)e^{-p^2}\, a(p)p_z + \frac{1}{\pi}\int e^{-p_1^2}\, a(p_1)p_{z1}\left[R - \left(\frac{2}{R}\right)e^{\omega^2}\right]d\mathbf{p}_1 = \left(p^2 - \frac{5}{2}\right)p_z. \tag{7}$$

Similarly, the coefficient of viscosity μ for a simple gas is given by

$$\mu = \frac{8}{15\pi\sigma^2}\sqrt{2mkT}\int_0^\infty e^{-p^2}\ \ b(p)p^6\ dp, \tag{8}$$

$b(p)$ being a solution of

$$M(p)e^{-p^2}\, b(p)p_x p_y + \frac{1}{\pi}\int b(p_1)e^{-p_1^2}\, p_{x1}p_{y1}\left[R - \left(\frac{2}{R}\right)e^{\omega^2}\right]d\mathbf{p}_1 = p_x p_y. \tag{9}$$

Evidently, equation (7) involves only the spherical harmonic of order 1 and equation (9) only the spherical harmonic of order 2. Likewise, the evaluation of the coefficient of diffusion D involves only the spherical harmonic of order 1.

We are therefore led to consider the expansion of the kernel $[R - (2/R)e^{\omega^2}]$ into spherical harmonics of the angle θ' between $\mathbf{p}$ and $\mathbf{p}_1$, the coefficients of the first and second harmonic being sufficient for the evaluation of λ, D, and μ, respectively. Putting

$$\left[R - \left(\frac{2}{R}\right)e^{\omega^2}\right] = \sum_{n=0}^{\infty}\left(n + \frac{1}{2}\right)A_n(p, p_1)P_n(\cos\theta'), \tag{10}$$

we have to evaluate

$$A_n(p, p_1) = \int_0^\pi \sin\theta'\, d\theta'\left[R - \left(\frac{2}{R}\right)e^{\omega^2}\right]P_n(\cos\theta'). \tag{11}$$

Now

$$\int_0^\pi RP_n(\cos\theta')\sin\theta'\,d\theta' = \frac{2p}{(2n+1)}\left[\frac{1}{(2n+3)}\left(\frac{p}{p_1}\right)^{n+1} - \frac{1}{(2n-1)}\left(\frac{p}{p_1}\right)^{n-1}\right], \quad p_1 > p, \tag{12}$$

$$= \frac{2p_1}{(2n+1)}\left[\frac{1}{(2n+3)}\left(\frac{p_1}{p}\right)^{n+1} - \frac{1}{(2n-1)}\left(\frac{p_1}{p}\right)^{n-1}\right], \quad p_1 < p. \tag{13}$$

Let

$$H_n(p, p_1) = \int_0^\pi P_n(\cos\theta')\sin\theta'\left(\frac{1}{R}\right)\exp\left(\frac{p^2p_1^2\sin^2\theta'}{R^2}\right)d\theta' = e^{p_1^2}\,t_n(p, p_1); \tag{14}$$

then, since $H_n(p, p_1)$ is symmetrical in p and p_1, it will be sufficient to evaluate $t_n(p, p_1)$ for the case $p_1 < p$ only. This can be accomplished by the following transformation:

$$t_n(p, p_1) = \int_{-1}^{1}\frac{P_n(x)}{R}\,e^{-z^2}\,dx =$$

$$\frac{1}{pp_1}\int_{-p_1}^{p_1}\left(1 + \frac{z}{\sqrt{z^2+a^2}}\right)P_n\left[\frac{1}{pp_1}\left(p_1^2 - z^2 - z\sqrt{z^2+a^2}\right)\right]e^{-z^2}\,dz, \tag{15}$$

where

$$a^2 = p^2 - p_1^2, \qquad z = \frac{1}{2}\left(R - \frac{a^2}{R}\right), \qquad R = (z + \sqrt{z^2+a^2}),$$
$$x = \cos\theta' = \frac{1}{pp_1}\left(p_1^2 - z^2 - \sqrt{z^2+a^2}\right). \tag{16}$$

We note that only even powers of z in the integrand of equation (15) will contribute to the integral, so that terms containing the radical will drop out. Putting $n = 0$, 1, 2 in equations (13) and (15), we get

$$pp_1A_0(p, p_1) = \frac{2}{3}p_1^3 + 2p^2p_1 - 4P(p_1), \qquad p_1 < p, \tag{17}$$

$$p^2p_1^2A_1(p, p_1) = \frac{2}{15}p_1^5 - \frac{2}{3}p_1^3p^2 - 4g(p_1), \qquad p_1 < p, \tag{18}$$

$$p^3p_1^3A_2(p, p_1) = \left[\frac{2}{35}p_1^7 - \frac{2}{15}p_1^5p^2 + 3p^2p_1 - 3p_1^3 + 18p_1 + (-6p_1^4 + 2p^2p_1^2 - 3p^2 + 15p_1^2 - 18)P(p_1)\right], \quad p_1 < p, \tag{19}$$

where

$$g(p) = [p + (p^2 - 1)P], \qquad P = e^{p^2}\int_0^p e^{-x^2}\,dx. \tag{20}$$

When $p_1 > p$, we must interchange p with p_1 in the right-hand sides of equations (17), (18), and (19).

Our method of solution consists in *reducing the Boltzmann-Hilbert integral equation to an ordinary differential equation.* The procedure will be illustrated in the following section for the case of self-diffusion.

3. *The Coefficient of Self-diffusion.*—We consider a gas consisting of molecules of mass m, diameter σ, and number density n. The molecules are divided into two groups which are somehow labeled but which are otherwise mechanically indistinguishable. Let the number density of the molecules of the first group be $n_1(\mathbf{r})$, that of the second group $n_2(\mathbf{r})$; then, since their sum, $n = [n_1(\mathbf{r}) + n_2(\mathbf{r})]$, is assumed to be uniform, we have $\partial n_1/\partial \mathbf{r} = -\partial n_2/\partial \mathbf{r}$. We wish to determine the rate of self-diffusion of the two groups. The equilibrium distributions in each group are given by

$$f_1^0 = n_1 a e^{-bc_1^2}, \qquad f_2^0 = n_2 a e^{-bc_2^2}; \tag{21}$$

$$a = \left(\frac{m}{2\pi kT}\right)^{3/2}, \qquad b = \frac{m}{2kT}. \tag{22}$$

Due to diffusion, these equilibrium distributions will be modified to

$$f_1 = f_1^0(1 + \varphi_1), \qquad f_2 = f_2^0(1 + \varphi_2), \tag{23}$$

where a suitable form for the φ's is found to be

$$\varphi_1(\mathbf{c}_1) = -\frac{1}{n_1}\frac{\partial n_1}{\partial \mathbf{r}}\cdot\mathbf{c}_1\Delta(c_1); \qquad \varphi_2(\mathbf{c}_2) =$$

$$-\frac{1}{n_2}\frac{\partial n_2}{\partial \mathbf{r}}\cdot\mathbf{c}_2\Delta(c_2) = +\frac{1}{n_2}\frac{\partial n_1}{\partial \mathbf{r}}\cdot\mathbf{c}_2\Delta(c_2), \tag{24}$$

$\Delta(c)$ being a function of the absolute value of c which is to be determined from Boltzmann's integral equation. This integral equation applies to f_1 and f_2 separately:[8]

$$\mathfrak{D}f_1^0 = -n_1^2 I_1(\varphi_1) - n_1 n_2 I_{12}(\varphi_1 + \varphi_2) = \left(\frac{1}{n_1}\right) f_1^0 \mathbf{c}_1 \cdot \frac{\partial n_1}{\partial \mathbf{r}}, \tag{25}$$

$$\mathfrak{D}f_2^0 = -n_2^2 I_2(\varphi_2) - n_1 n_2 I_{21}(\varphi_1 + \varphi_2) = \left(\frac{1}{n_2}\right) f_2^0 \mathbf{c}_2 \cdot \frac{\partial n_2}{\partial \mathbf{r}}, \tag{26}$$

where the integral operators I are defined by

$$n_1^2 I_1(F) = \frac{\sigma^2}{2}\iint f_1^0 f^0 (F_1 + F - F_1' - F')\,|\,\mathbf{g}\cdot\mathbf{k}\,|\,d\mathbf{k}d\mathbf{c}, \tag{27}$$

$$n_1 n_2 I_{12}(K) = \frac{\sigma^2}{2}\iint f_1^0 f_2^0 (K - K')\,|\,\mathbf{g}\cdot\mathbf{k}\,|\,d\mathbf{k}d\mathbf{c}_2, \tag{28}$$

F being a function of $\mathbf{c}_1$ and K a function of $\mathbf{c}_1$ and of $\mathbf{c}_2$. Either equation (25) or equation (26) suffices for the determination of $\Delta(c)$. Using Hilbert's transformation, we get

$$n_1^2 I_1(\varphi_1) = \frac{n_1^2\sigma^2 m}{2\pi kT}\Big\{M(p_1)e^{-2p_1^2}\varphi_1(p_1) + \left(\frac{1}{\pi}\right)e^{-p_1^2}\int e^{-p_2^2}\varphi_1(p_2)\left[R - \left(\frac{2}{R}\right)e^{\omega^2}\right]d\mathbf{p}_2\Big\}, \quad (29)$$

$$n_1 n_2 I_{12}(\varphi_1 + \varphi_2) = \left(\frac{n_1 n_2\sigma^2 m}{2\pi kT}\right)M(p_1)e^{-2p_1^2}\varphi_1(p_1) + \left(\frac{n_1 n_2\sigma^2 m}{2\pi^2 kT}\right)e^{-p_1^2}\int e^{-p_2^2}\Big\{\varphi_2(p_2)R - \left(\frac{1}{R}\right)e^{\omega^2}[\varphi_1(p_2) + \varphi_2(p_2)]\Big\}d\mathbf{p}_2. \quad (30)$$

Now it follows from equation (24) that

$$n_2\varphi_2(p_2) = -n_1\varphi_1(p_2). \quad (31)$$

Substituting equations (24) and (30) in (25), and using equation (31), we get

$$\left(\frac{1}{n_1}\right)f_1^0\mathbf{c}_1\cdot\frac{\partial n_1}{\partial \mathbf{r}} = \frac{n_1 n\sigma^2 m}{2\pi kT}\Big[-M(p_1)e^{-2p_1^2}\varphi_1(p_1) + \left(\frac{1}{\pi}\right)e^{-p_1^2}\int e^{-p_2^2+\omega^2}\left(\frac{1}{R}\right)\varphi_1(p_2)\,d\mathbf{p}_2\Big], \quad (32)$$

where $\omega^2 = (p_1^2 + p_2^2 - 2p_1p_2\cos\theta')$.

It will be convenient to adopt a spherical system of co-ordinates in $\mathbf{p}_1$, with the polar axis pointing in the direction of ∇n_1. Then

$$n_1\varphi_1(\mathbf{c}_1) = -c_1\Delta(c_1)\cos\theta_1\left|\nabla n_1\right| = -\left(\frac{1}{\sigma^2 n\sqrt{\pi}}\right)p_1\delta(p_1)\cos\theta_1\left|\nabla n_1\right|, \quad (33)$$

where

$$\delta(p_1) = \sigma^2 n\sqrt{\frac{2\pi kT}{m}}\,\Delta(p_1), \quad (34)$$

and equation (32) becomes

$$p_1\cos\theta_1 = M(p_1)e^{-p_1^2}p_1\delta(p_1)\cos\theta_1 - \left(\frac{1}{\pi}\right)\int e^{-p_2^2+\omega^2}\left(\frac{1}{R}\right)p_2\delta(p_2)\cos\theta_2\,d\mathbf{p}_2. \quad (35)$$

By equations (14) and (15), the kernel of this integral equation can be analyzed into spherical harmonics, of which only the harmonic of order 1 enters into equation (35):

$$\int e^{-p_2^2+\omega^2}\left(\frac{1}{R}\right)p_2\delta(p_2)\cos\theta_2\,d\mathbf{p}_2 = 2\pi\cos\theta_1\int_0^\infty e^{-p_2^2}H_1(p_1, p_2)\delta(p_2)p_2^3\,dp_2, \quad (36)$$

where

$$H_1(p_1, p_2) = \int_0^\pi P_1(\cos\theta') \sin\theta' \, d\theta' \left(\frac{1}{R}\right) e^{\omega^2} = \left(\frac{2}{p_1^2 p_2^2}\right) g(p_1), \qquad p_1 < p_2, \quad (37)$$

$$= \left(\frac{2}{p_1^2 p_2^2}\right) g(p_2), \qquad p_1 > p_2. \quad (38)$$

Hence the Boltzmann-Hilbert integral equation, in the case of self-diffusion, takes on the form

$$e^{-p^2} M(p) p^3 \delta(p) - p^3 = 4 \int_0^p e^{-x^2} g(x)\delta(x) x \, dx + 4g(p) \int_p^\infty e^{-x^2} \delta(x) x \, dx. \quad (39)$$

The coefficient of self-diffusion D_{11} is defined by

$$-D_{11}\left(\frac{1}{n_1}\frac{\partial n_1}{\partial \mathbf{r}} - \frac{1}{n_2}\frac{\partial n_2}{\partial \mathbf{r}}\right) = \frac{1}{n_1}\int f_1 \mathbf{c}_1 \, d\mathbf{c}_1 - \frac{1}{n_2}\int f_2 \mathbf{c}_2 \, d\mathbf{c}_2, \quad (40)$$

which leads to the result

$$D_{11} = \alpha \frac{3}{8n\sigma^2}\sqrt{\frac{kT}{\pi m}}, \quad (41)$$

$$\alpha = \frac{64}{9\sqrt{2\pi}} \int_0^\infty e^{-p^2} \delta(p) p^4 \, dp. \quad (42)$$

The integral equation (39) will now be reduced to a differential equation. Put

$$e^{-p^2}\delta(p) = \Psi(p), \qquad \int_p^\infty \Psi(x) x \, dx = f(p) \quad (43)$$

in equation (39), obtaining

$$-p^2 M(p) \left(\frac{df}{dp}\right) - p^3 = 4g(p)f(p) - 4\int_0^p g(x)\dot{f}(x)dx = 4\int_0^p f(x)\dot{g}(x) \, dx, \quad (44)$$

$$\frac{d}{dp}\left[p^2 M(p)\dot{f}\right] + 4\dot{g}f = -3p^2, \quad (45)$$

which is an inhomogeneous second-order ordinary differential equation for the determination of $f(p)$. Similarly, one can derive from equation (39) the differential equation for $\Psi(p)$,

$$\frac{d}{dp}\left[\frac{1}{\dot{g}}\frac{d}{dp}(p^3 M\Psi)\right] + 4p\Psi(p) = 3\frac{d}{dp}\left(\frac{p^2}{\dot{g}}\right). \quad (46)$$

We have integrated numerically the differential equation (46), and some of the values are given in Table 1 under $\Psi_2(p)$. This solution yields

$$\int_0^\infty \Psi(p) p^4 \, dp = 0.35918, \qquad \alpha = 1.01896. \quad (47)$$

4. *Discussion of Results.*—The values of α obtained in the successive approximations of the Chapman-Enskog method[9] are 1, 1.005, 1.010, 1.019, the final value agreeing with equation (47).

The problem of self-diffusion in a gas model of rigid spheres was treated by Pidduck,[5] who based his analysis on Hilbert's transformation. Pidduck arrives at an integral equation[10]

$$\Theta_1(p) - \int_0^\infty k_1(p, p')\Theta_1(p')\,dp' = pG_1(p), \tag{48}$$

for the determination of $\Psi(p)$, where

$$\Theta_1(p) = \frac{p^2\sqrt{M(p)}\Psi(p)}{n\sigma^2\sqrt{\pi}}, \qquad G_1(p) = \frac{p}{\sqrt{\pi M(p)}n\sigma^2}, \tag{49}$$

$$k_1(p, p') = \frac{4\exp\left[\frac{1}{2}(p^2 + p'^2)\right]}{pp'\sqrt{M(p)\cdot M(p')}}\int_y^x e^{-[\theta^2+(x^2y^2/\theta^2]}(x^2 + y^2 - 2\theta^2)\,d\theta, \tag{50}$$

$$x = \frac{1}{2}(p + p'), \qquad y = \frac{1}{2}|p - p'|. \tag{51}$$

Pidduck evaluates the kernel $k_1(p, p')$ by integrating equation (50) numerically for a double series of values of p and p' situated in the octant of the (p, p')-plane where $p > p'$, except for the case $p = p'$, for which

$$k_1(p, p) = \frac{4g(p)}{p^2M(p)}. \tag{52}$$

He then iterates this kernel numerically and achieves convergence for the solving kernel after the fifth iteration.

The kernel $k_1(p, p')$ can be evaluated explicitly with the aid of transformation (15):

$$k_1(p, p') = \frac{4g(p')}{pp'\sqrt{M(p)\cdot M(p')}}, \qquad p' < p, \tag{53}$$

$$= \frac{4g(p)}{pp'\sqrt{M(p)M(p')}}, \qquad p' > p. \tag{54}$$

Table 1 shows a comparison of the values of $\Psi_1(p)$ obtained by Pidduck[5] with the values $\Psi_2(p)$ obtained by the author by integrating the second-order differential equation (46) for $\Psi(p)$.

TABLE 1

COMPARISON OF $\Psi_1(p)$ COMPUTED BY PIDDUCK BY SOLVING NUMERICALLY THE INTEGRAL EQUATION (48), WITH THE VALUES $\Psi_2(p)$ OBTAINED BY THE AUTHOR BY INTEGRATING NUMERICALLY THE SECOND-ORDER DIFFERENTIAL EQUATION (46) FOR $\Psi(p)$

p	$\Psi_1(p)$	$\Psi_2(p)$	p	$\Psi_1(p)$	$\Psi_2(p)$	p	$\Psi_1(p)$	$\Psi_2(p)$
0.1	0.630	0.69956	1.0	0.223	0.22467	1.9	0.0131	0.01311
0.2	0.635	0.67525	1.1	0.177	0.17765	2.0	0.00867	0.00865
0.3	0.613	0.63671	1.2	0.137	0.13761	2.1	0.00564	0.00560
0.4	0.575	0.58662	1.3	0.103	0.10444	2.2	0.00358	0.00356
0.5	0.529	0.52825	1.4	0.0780	0.07767	2.3	0.00222	0.00221
0.6	0.451	0.46508	1.5	0.0565	0.05662	2.4	0.00134	0.00135
0.7	0.397	0.40049	1.6	0.0405	0.04046	2.5	0.00082	0.00081
0.8	0.338	0.33743	1.7	0.0284	0.02834	2.6	0.00047	0.00047
0.9	0.277	0.27826	1.8	0.0194	0.01946	2.7	0.00027	0.00027

After this investigation was finished, the author discovered that Boltzmann, in a paper on self-diffusion,[11] had arrived at the integral equation (39) and had then reduced this integral equation to a differential equation[12] which is identical with equation (45). This fact appears not to have been known either to Hilbert or to Pidduck. Boltzmann derives the power-series solution of equation (45) for small p, then obtains an asymptotic series valid for large p, and also indicates how to proceed with the integration for intermediate values of p. However, he does not attempt to integrate this differential equation numerically, a step which would have yielded him a value for the coefficient of self-diffusion—this in spite of the 63 pages of rather heavy mathematics which he devotes to the diffusion problem.

The method by which Boltzmann arrives at the integral equation (39) originates in his study of viscosity.[13] There he proceeds to obtain a power-series solution for the unknown function directly from his integral equation (1), a task which is very laborious and which extends over 160 pages. In the course of this work he recognizes[14] that certain component series in his solution can be summed in terms of the exponential integral. Toward the end of this investigation he obtains the simplified form of the integral equation (9) which one can derive[15] by the use of equation (19) and then reduces it to an ordinary differential equation of the fourth order, but, again, he fails to integrate it. Had he carried out the numerical integration, he would have obtained a value for the coefficient of viscosity, which was the purpose of his investigation, and he would have been able to summarize these efforts on a more happy tone than "Boltzmann attempts to solve exactly by this method the typical cases of viscosity and diffusion on the assumption that the molecules are elastic spheres; [he] is, however, led thereby to very complicated series-expansions which are hardly suited for practical computation, or to a differential equation of the fourth order whose coefficients are transcendental functions."[16]

5. *Summary.*—The Boltzmann-Hilbert integral equation is the simplified form of Boltzmann's integral equation for the velocity distribution function which Hilbert derived for the case of a model gas of rigid spheres. In this paper a transformation is given by which one can obtain the expansion of the kernel of the Boltzmann-Hilbert integral equation into spherical harmonics, and the coefficients of the harmonics up to the second are evaluated explicitly. With the aid of these, one can simplify the Boltzmann-Hilbert integral equation, in the case of viscosity, heat conduction, and self-diffusion of a simple gas, to an integral equation in one dimension. This integral equation can be solved by reducing it to an ordinary differential equation. The method is illustrated in this paper for the case of self-diffusion, where the resulting differential equation is of the second order. This differential equation is integrated numerically, and the value of the coefficient of self-diffusion obtained agrees very well with the value obtained by the Chapman-Enskog method. The work is then compared with the treatment of self-diffusion by Pidduck, which was also based on Hilbert's form of the integral equation. Subsequently it was found that Boltzmann had derived, by very laborious methods, the second-order differential equation for the case of self-diffusion but failed to integrate it. This is true also of his work on viscosity, where the differential equation is of the fourth order. Had he carried out the numerical integration of these ordinary differential equations, he would have determined the values of the

coefficients of self-diffusion and viscosity, whereby his efforts on these problems, covering 231 pages of his *Collected Works*, would have borne fruit.

[1] L. Boltzmann, *Collected Works* (1872), **1**, 361.

[2] D. Hilbert, *Grundzüge einer allgemeinen Theorie der linearen Integralgleichungen* (New York: Chelsea Publishing Co., 1953), p. 267.

[3] S. Chapman and T. G. Cowling, *The Mathematical Theory of Non-uniform Gases* (Cambridge: At the University Press, 1952).

[4] S. Boguslawski, *Math. Ann.*, **76**, 431, 1915.

[5] F. B. Pidduck, *Proc. London Math. Soc.*, **15**, 89, 1916.

[6] S. Chapman, *Phil. Trans. Roy. Soc.*, **A216**, 279, 1916; **A217**, 115, 1917.

[7] D. Enskog, dissertation, Uppsala, 1917.

[8] Chapman and Cowling, *op. cit.*, pp. 85 and 139.

[9] *Ibid.*, p. 198.

[10] Pidduck, *op. cit.*, p. 99.

[11] Boltzmann, *op. cit.*, **3**, 48.

[12] *Ibid.*, eq. (100) where y is proportional to Ψ.

[13] *Ibid.*, **2**, 388–556.

[14] *Ibid.*, p. 541.

[15] See a forthcoming publication by the author.

[16] L. Boltzmann and J. Nabl, *Encyklopedie math. Wiss.*, vol. V, Part 1, p. 541, 1905.

EXPERIMENTAL REVERSAL OF SEX IN THE GONADS OF THE OPOSSUM DIDELPHIS VIRGINIANA

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Since steroid sex hormones in pure form became available for experimental purposes some twenty years ago, much work has been devoted to the study of their effects on the developing genital system in the embryo and the problem of the role of sex hormones in the normal differentiation of sex. In many species of amphibia, and in bird embryos, sex hormones administered during the period of sexual differentiation readily control the development of the accessory sex structures; at the same time they also transform the gonads histologically by reversing the normal balance (as genetically determined) between the medullary and cortical components.

In the case of mammalian embryos, however, a serious discrepancy has long existed in the apparent failure of sex hormones to induce significant histological changes in the gonads, even in cases in which the accessory sex structures are strongly transformed. This was the situation encountered in several species of placental mammal (rat, mouse, rabbit, guinea pig, hamster, and rhesus monkey) and in a marsupial—the North American opossum (*Didelphis virginiana* Kerr).[1] This conspicuous failure to influence the differentiation of mammalian gonads lent weight to the argument that the steroid sex hormones are probably not similar, either chemically or physiologically, to the sex-differentiating substances elaborated by the embryonic gonads and have no proper role in the normal processes of sex differentiation.

However, there seem to be no obvious reasons for supposing that mammalian gonads, as a class, are inherently incapable of reacting to sex hormones. On the contrary, indirect evidence indicates that—as in other vertebrates—the embryonic gonad of mammals is potentially bisexual in its constitution. Spontaneously occurring hermaphroditism, characterized by the development of ovotestes (hermaphroditismus verus), is a familiar anomaly in many mammals. Also, embryonic gonads which develop as grafts in strange environments frequently give rise to both ovarian and testicular tissues, through the unregulated development of both primordial sex components.[2] Finally, the modified ovaries of freemartins commonly develop testicular structure to a high degree.[3] There is, then, no reason to doubt that in the early stages of development mammalian gonads possess the requisite structural basis and are potentially capable of undergoing sex transformation. Their failure to react to sex hormones administered experimentally thus posed a problem.

The question remained in this state until a few years ago when a number of cases of well-developed ovotestes appeared in a litter of young opossums which had been treated from birth with a rather low dosage of the female hormone *estradiol dipropionate*.[4] In every male of this litter (seven in all) a well-defined cortical layer had developed over the surface of the testis, external to the tunica albuginea. The character of the induced cortex, and the state of preservation of the testicular region of these gonads, varied considerably in individual cases according to the length of treatment. The history of this experiment revealed that the litter had been born at an earlier stage than usual (this point will be considered later) and presumably had received the first hormone treatment earlier than in any previous experiment. Since at the time of birth the gonads of young opossums are just at the verge of histological sex differentiation, it seemed likely that the condition of the gonads when first exposed to the hormone might be the determining factor. However, the dosage was much lower than in previous experiments (1–2 μg. per day), and it was felt that this also might be important.

A histological study of the intersexual gonads showed that the first step in the differentiation of the cortical zone consists in a persistence of the *germinal epithelium* over the surface of the embryonic testis long beyond the stage when it normally disappears. This observation recalled certain cases noted occasionally in some of the earlier experiments on opossums, in which this phenomenon had occurred to a limited extent. Such cases were infrequent and were found only in subjects which were sacrificed (or had died) comparatively early; in older animals the germinal epithelium was absent or was so vestigial in character as to seem insignificant. It was now evident, however, that survival of any considerable area of germinal epithelium on a testis for an appreciable length of time represents, potentially at least, the initial stage in the development of a cortex. Thus these earlier cases were put in a new light.

This unexpected result, and the circumstances under which it appeared, suggested that previous failures—in the opposum at least—might perhaps be due to experimental conditions rather than to an innate incapacity of the embryonic gonads to react to sex hormones. If the embryos could be exposed to the hormone in the proper dosage and at a sufficiently early stage in development, transformation of the gonads might be obtained regularly. Further success seemed to hinge on

securing the young at the earliest possible moment after birth and, particularly, on the occasional appearance of litters born somewhat prematurely.

It is well established that young opossums are born after an unusually brief gestation of from 12.5 to 13 days. The evidence on this point has been reviewed by McCrady.[5] According to the study of McCrady, birth normally occurs at about 12 days and 19 hours, and in his series of normal stages, based on criteria of external form, the stage of birth is designated as *stage 35*. It is readily distinguished from *stage 34* (which precedes it by about 12 hours) by the form and proportions of the head, the structure of the hind limb, and especially by the *absence of the oral shield*—a peculiar plaque-like, epidermal structure surrounding the mouth which characterizes stage 34. In the case of the experimental litter which yielded the group of ovotestes referred to above, it is known (from specimens preserved and photographed when the litter was first seen) that the young were born at stage 34; hence they were probably treated some 12 hours earlier (approximately) than in previous experiments.

Accordingly, an effort has been made during the past three years (1) to secure embryos for treatment at the earliest possible moment after birth and (2) to determine how much variability may exist as to the stage at which viable embryos are born, since presumably only those born earlier than usual (at or near stage 34?) would provide favorable material for the purpose at hand. The effort has been rewarded to the extent that four other litters of this age have been obtained and used experimentally with success. The development of ovotestes in males after treatment with *estradiol dipropionate* has been confirmed. In a number of cases, moreover, the reversal process has gone much further than in the original group, the transformed gonads closely resembling ovaries in their structure and appearance. At the present time a total of twenty-three cases, representing every male in each of five litters, are available for study and are listed in Table 1.

TABLE 1

Litter	Length of Treatment (Days)	Av. Dose per Day (μg.)	Condition of the Cortex	No. of Cases
I	30	1–2	Well developed but *sterile;* persistent germinal epithelium	7
II	20	5	Thin to well developed but *sterile;* persistent germinal epithelium	6
III	10	0.3	Persistant germinal eipthelium; incipient cortical cords; *germ cells*	3
IV	10	0.2	Persistent germinal epithelium; incipient cortical cords; *germ cells*	2
V	20	0.15	Thick and well developed, with *germ cells;* persistent germinal epithelium; primordial follicles with growing ovocytes	5
			Total	23

The process of sex reversal in these gonads, as seen histologically, involves the gradual development of a cortical layer over the surface of an embryonic gonad which (as revealed by certain peculiarities of its later structure) has already undergone a certain amount of differentiation as a testis. The development of the cortical zone in an ovotestis is based, histologically, on the normal mechanism of sex differentiation. It is a well-established principle of sex differentiation in verte-

brates that up to a point in development the embryonic gonad—irrespective of its future sex as genetically determined—is potentially capable of becoming either an ovary or a testis. During the "sexually indifferent" phase of development this dual potentiality finds expression in the presence of two distinct histological elements (Fig. 1, *B*). The *primary sex cords*, which have originated somewhat earlier as masses of cells proliferated from the inner surface of the *germinal epithelium*, represent the male element of the indifferent gonad and are strictly male in potentiality. In the development of a testis they eventually form the *seminiferous*

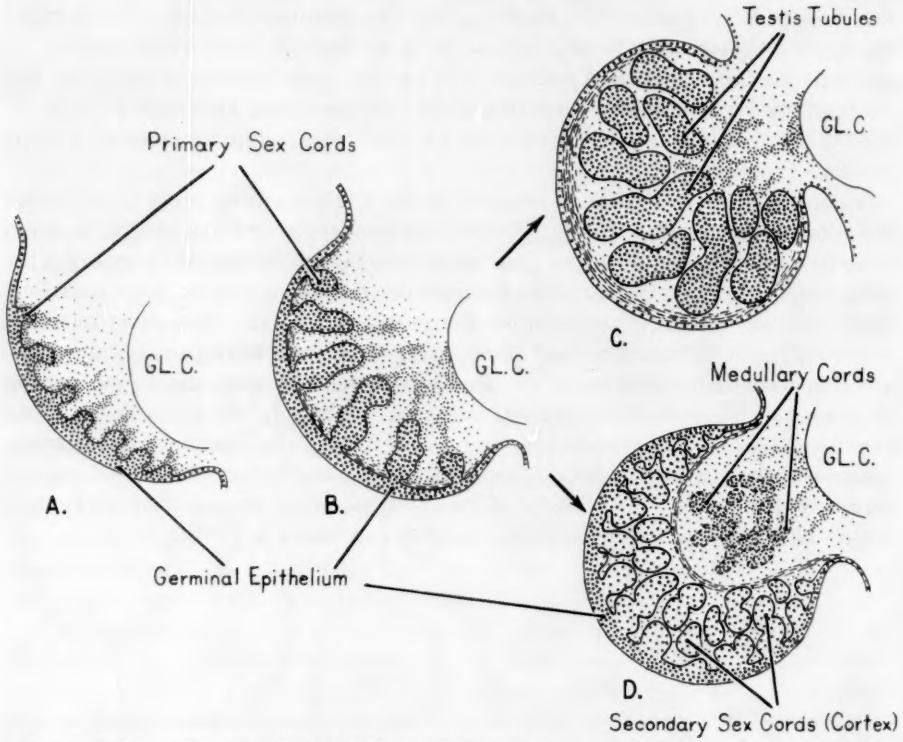


FIG. 1.—The process of sex differentiation in the gonads of mammalian embryos, illustrated semi-diagrammatically. *A:* The origin of the primary sex cords from the *germinal epithelium*. *B:* The *indifferent stage* of sex differentiation, in which the male, or testicular, component of the gonad is represented by the *primary sex cords;* the *germinal epithelium*, with its potentiality for producing *secondary sex cords*, represents the female component. *C:* The development of a testis—the *primary sex cords* become the testis tubules, while the germinal epithelium undergoes involution. *D:* The development of an ovary—*secondary sex cords* arise from the germinal epithelium form the cortex, while the primary sex cords retrogress. (In each drawing *GL. C.* is the cavity of a glomerular capsule of the mesonephros.)

tubules, while the germinal epithelium undergoes involution (Fig. 1, *C*). Female potentiality in the sexually indifferent phase resides in the germinal epithelium itself, which at this stage is present and well developed in both sexes. In prospective ovaries (Fig. 1, *D*) the germinal epithelium persists and continues to proliferate cells which give rise to the *secondary sex cords* and the *cortex* of the ovary. The primary sex cords (male component) regress and ultimately either disappear or survive in a more or less vestigial condition, their behavior varying somewhat in different species.

The various stages of reversal, as seen in the gonads of experimental animals, are patterned closely on this scheme of development, with the difference that under the influence of the female hormone a prospective testis is redirected into the pathway prescribed for the ovary. The first step in the reversal process must take place while the yount testis is still in the indifferent stage (Fig. 1, *B*). It consists in the survival and continued growth of the germinal epithelium of the testis after the stage at which it should normally disappear. Persistence of the germinal epithelium is a striking feature in all ovotestes. At the same time the testis proper is inhibited—it becomes greatly reduced in size, while the primary sex cords (prospective testis tubules) are distinctly atrophic. An ovotestis in such an early stage of reversal is shown in Figure 2. At an age of 20 days *post partum* the germinal epithelium is well developed over the entire surface of the testis external to the tunica albuginea although it would normally have disappeared completely within a few days after birth. In many places clusters of cells proliferating from the inner surface of the germinal epithelium represent the beginnings of secondary sex cords—the cortical component of the ovotestis (Figs. 2 and 3).

In specimens treated for a longer time (30 days) with female hormone the production of cortical cords continues until a thick and compact layer of tissue underlies the germinal epithelium. The cortex thus formed is always external to the fibrous tunic layer, which separates it everywhere from the testicular regions of the gonad (Figs. 3 and 4). Thus there can be no question as to the origin of the cortical zone of the ovotestis: it is the product of the persistent germinal epithelium, developing in exactly the same way as does the cortex of the normal ovary.

Many different stages in the development of the cortex, intermediate between the conditions shown in Figures 3 and 4, are found. In such a series, parallel stages in the atrophy and transformation of the primary sex cords are encountered, until, in cases with a well-developed cortex like that in Figure 4, little evidence remains of tubular structure. They are finally reduced to the status of pale-staining, epithelial strands with no trace of a lumen and in this condition closely resemble their homologues—the so-called "medullary cords" of the ovary.

At this point an important feature of these intersexual gonads must be emphasized—the total absence of germ cells in the induced cortex (compare Figs. 4 and 5). Complete sterility of the cortex was found in all ovotestes of the first two litters of Table 1. The reason remains uncertain, but is is possibly related to the dosage. In the later experimental groups, in which considerably lower dosages were used, it did not occur, although the number of germ cells present was much lower than that in the cortex of a normal ovary. Cortical sterility, then, is not a necessary condition in transformed testes.

By far the most complete and convincing examples of histological transformation are found in the males of the last three experimental litters, which received the lowest dosages of the hormone yet administered (Table 1). In these animals the internal organs of sex were all strongly transformed, and the copulatory organs were of female type and greatly hypertrophied. Obviously, from the stand point of the accessory sex structures, the dosages in these experiments are still well above the level required to elicit a strong reaction. The gonads of these males can no longer be properly called ovotestes; indeed, in most respects they approach very closely the structure of ovaries. The development of the cortex is remarkable, far exceeding anything previously encountered (Fig. 5). The germinal epithelium

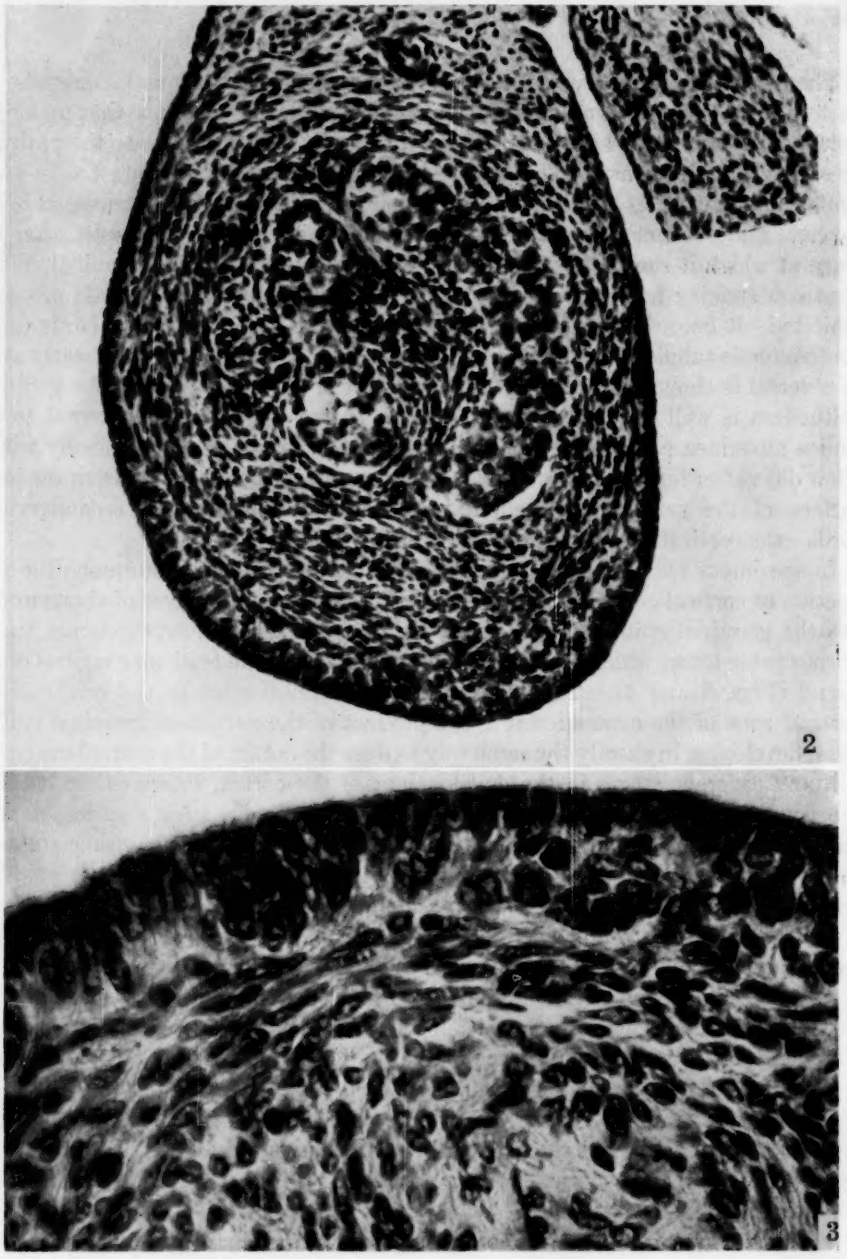
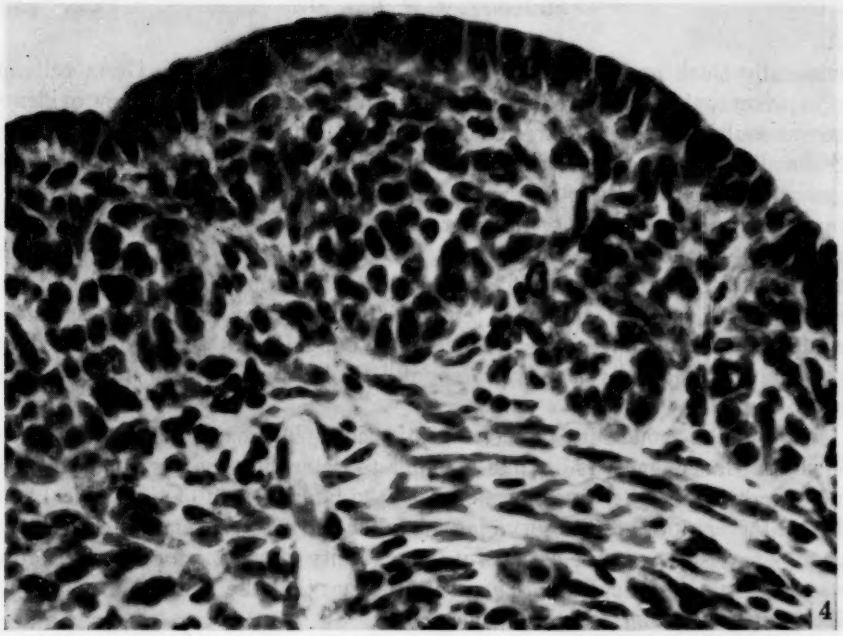


PLATE I

FIG. 2.—Photomicrograph of an ovotestis in a young male opossum (litter II, Table 1) produced by treatment with female hormone for a period of 20 days. The testis cords (*primary sex cords*) are greatly reduced in size and show evidences of degeneration. The persistent *germinal epithelium* covers the entire surface of the gonad and shows marked thickening over the distal region (*below*), where *secondary sex cords* are beginning to form. The incipient cortex is everywhere separated from the testicular part of the gonad by a thick fibrous layer, the *tunica albuginea*. ×200.

FIG. 3.—Photomicrograph, at higher power, of the incipient cortex of the ovotestis shown in Figure 2. Prominent clusters of cells, proliferating from the undersurface of the *germinal epithelium*, represent the *secondary sex cords*. The underlying layer of flattened cells is the *tunica albuginea*. ×600.



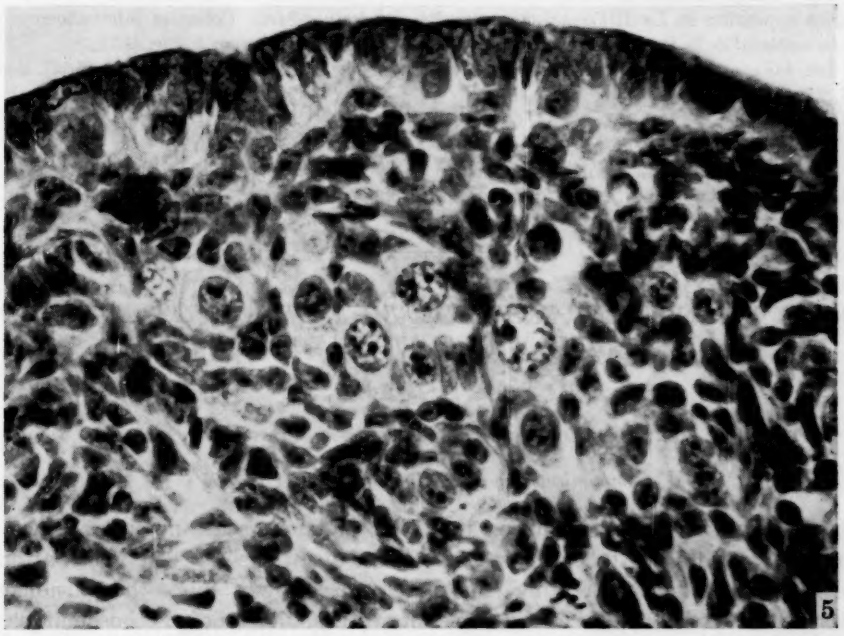


PLATE II

FIG. 4.—The cortical zone and the underlying *tunica albuginea* in an ovotestis, after treatment with female hormone for 30 days (litter I, Table 1). The cortex is composed of numerous cortical cords, compactly arranged, external to the *tunica albuginea* however, no germ cells appear, either in the cords or in the *germinal epithelium*. Compare with Figure 5. ×500.

FIG. 5.—Photomicrograph showing the cortical region of a transformed testis in a member of litter V. The structure is typically ovarian. Germ cells are present throughout the cortex, but in greatly reduced numbers as compared with a normal ovary. ×600.

is unusually thick and covers the entire surface of the gonad. Germ cells are always present, although in reduced numbers; moreover, they often show evidences of recent multiplication and are rather evenly distributed throughout the cortex. Occasionally, well-developed primordial follicles containing ovocytes in early growth stages are found. On the other hand, certain defects or peculiarities of structure always remain to indicate the testicular origin of these gonads. The reduction in the number of germ cells in the cortex has been mentioned, and the medullary (original testicular) region is always more extensive than would be found in a normal ovary. In some specimens remnants of testis tubules, and rete structures of male type, have survived in the medullary one adjacent to the hilum. However, such features are relatively minor defects in gonads which are essentially ovaries.

Certain points remain for further investigation. The experiments must be extended over longer periods of time in order to determine the permanence of the changes induced. Other questions, such as the later history of the cortical germ cells and the final fate of the surviving medullary structures, remain to be settled. This may require some time because of the necessity of waiting for litters of proper age and because of difficulties arising from the fact that the female hormone is not well tolerated over long periods of time.

[1] The results for most of the species mentioned are discussed and summarized in a series of articles appearing in *La Différenciation sexuelle chez les vertébrs: Colloques internationaux du Centre national de la Recherche scientifique*, Vol. XXXI (Paris: Masson & Cie, 1951).

[2] See, for example, A. Buyse, *J. Exptl. Zool.*, **70**, 1–41, 1935; and T. Torrey, *J. Exptl. Zool.*, **115**, 37–58, 1950.

[3] B. H. Willier, *J. Exptl. Zool.*, **33**, 63–127, 1921.

[4] R. K. Burns, *Arch. anat. microscop. et morphol. exptl.*, **39**, 467–483, 1950.

[5] E. McCrady, Jr., *The Embryology of the Opossum* ("American Anatomical Memoirs, No. 16 Philadelphia: Wistar Institute of Anatomy and Biology, 1938).

SOCIAL BEHAVIOR IN FRESH-WATER FISH AND ITS EFFECT ON RESISTANCE TO TRYPANOSOMES

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In the course of experiments on the effect of temperature upon resistance of fresh-water fishes to trypanosome infection, unanticipated behavior patterns which modified the temperature effects were discovered. Since the phenomena observed are likely to be of considerable interest to students of psychosomatic and social aspects of resistance, these experiments are reported here in advance of a large body of data on fish trypanosomiasis obtained at the same time.

The fish in these experiments were kept in the constant-temperature apparatus of the zoölogy department of Cambridge University[1] in aquaria measuring 24 × 12 × 12 inches. The temperatures maintained were 5°, 10°, 15°, and 20° C., with aeration and a constant slow flow of the water. The fish were fed *Daphnia*, *Enchy-*

traeus, *Tubifex*, or chopped earthworms every other day, and it was possible to maintain as many as six fish that were 5 inches long in each aquarium. The following fish were used: (1) Ten tench (*Tinca tinca*) purchased from a dealer. They were very emaciated and heavily infected with trypanosomes and a skin infection of *Ichthyophthirius* (which disappeared two days after the fish were placed at 15° or 20° C.). (2) Ten perch (*Perca fluviatilis*) that were infected with trypanosomes. Six of these had been maintained in a running-water aquarium in the laboratory for eighteen months, while the other four were caught in the Cam River just after the ice thawed in the winter of 1954. (3) Six goldfish (*Carassius auratus*) purchased from a dealer in Cambridge. They were infected with trypanosomes by the bite of leeches which had previously fed on the infected perch from the laboratory stocks.

To examine the fish for trypanosomes, blood was obtained by clipping off small portions of gill filaments from the anesthetized fish. The blood was transferred by a small pipette to slides for smearing and staining or examining fresh.

Table 1 indicates which fish lose their infection, the conditions controlling this loss, the behavior of the fish, and the fish that were bled for antibodies. The perch and goldfish behaved in the same manner in all temperature conditions studied, with possibly more activity in the 20° C. tank. Their behavior was in the nature

TABLE 1*

EFFECT OF TEMPERATURE ON THE BEHAVIOR OF FISH AND ON THEIR RESISTANCE TO TRYPANOSOME INFECTIONS

KIND OF FISH	DURATION OF ARRANGEMENTS (DAYS)	TEMPERATURE OF AQUARIA (CENTIGRADE) 20°	15°	10°	5°
Perch	28	(1)†–(5)	6–7	2–3	4–8–9–10
Perch	7	(2)†–(3)†	6–7	4–8	9–10
Perch	28	(4)†–(8)	6–7	...	9–10
Perch	14	(6)–(10)	7–9	...	...
Tench	28	(1)† > 2‡–3‡	7–8	4–5–6	...
Tench	7	(2)† > 4‡–3‡	7–8	5–6	...
Tench	21	(4)† > 5‡–3‡	7–8	6	...
Tench	12	(5)†§	7–8	...	...
Tench	14	(3)†§	7–8	...	...
Goldfish	21	...	1–2–3	4–5–6	...
Goldfish	14	(1)–(2)–(3)	...	4–5–6	...
Goldfish	12	(4)–(5)	...	6	...

* Numbers indicate individual fish and are those used to follow their sequence throughout the experiments. Parentheses around numbers indicate that trypanosomes disappeared in the blood in 5–7 days. Dashes between numbers indicate peck-dominance relationship; fish to the left tended toward dominance in most cases. > indicates that fish to the left dominated territory.

† Tested for antibodies.

‡ Ceased feeding.

§ Placed in a battery jar at same temperature after 7 days.

of a "peck-dominance"[2, 3] with all the fish able to feed satisfactorily. Some of the perch were in the tanks with the tench for part of the experiments; no interference with behavior or feeding was observed between the two species, even in the 20° C. tank. Although quantitative determinations of trypanosomes in blood of the fish could not be made accurately because of the dilution of the blood sample by water on the gills, it was possible to distinguish any drastic reduction of the trypanosomes. It was observed that in all the perch and goldfish, after about a week in the 20° tank, the number of trypanosomes was drastically reduced. In some

specimens (perch Nos. 2 and 4; goldfish Nos. 2, 4, and 5) none could be found on the slides at all. Perch Nos. 1 and 8 were observed to maintain a very low infection of rare parasites for three weeks after the initial reduction. The fish in the other tanks retained high infections of trypanosomes throughout their stays in those tanks, only to lose them when transferred to the highest temperature.

The tench behaved similarly to the goldfish and perch in the 10° and 15° tanks: they all fed; their behavior showed a very loose "peck-dominance"; and the high infections of trypanosomes were retained. In the 20° C. aquarium the tench behaved quite differently. They became highly irritable, and the slightest jar of the aquarium, turning on of lights, or movement of other fish would send them all into frenzied dashes around the aquarium. Eventually the activity became great enough to disrupt the heating element in the tank. Moreover, their behavior exhibited marked territoriality.[2, 4] One tench dominated the whole territory and constantly drove the others into opposite corners of the tanks. The dominant fish was the only one that fed. It was also the only fish to eliminate its trypanosome infection. Tench No. 3 remained in the tank eight weeks without losing its infection, while No. 1 lost its infection within a week's time, after which period it was removed. After the dominant fish was removed, No. 4 was taken from the 10° C. tank and placed in the 20° C. tank. The fish that remained in the tank did not feed for a day or two after the dominant fish was removed. Fighting started after the introduction of the new fish, with fish No. 2 becoming dominant and eventually losing its infection of trypanosomes. The experiment was again repeated by replacing the dominant fish with a fish from a lower temperature, with the same result as described above. With the removal of the third dominant fish, the two remaining fish were left in the tank. Neither fed for a week, although living *Tubifex* were moving on the bottom of the tank. At the end of the week tench No. 5 immediately began feeding when placed in a battery jar floated in the 20° tank, and it had lost its infection by the fifth day under these conditions.

TABLE 2

EFFECT OF SERUM ON TRYPANPSOMES (10° C.)

Serum Source	Time since Loss of Trypanosomes Observed	Effect*
Tench 1	3 weeks	−
Tench 2	1–2 days	+++†
Tench 3	1–2 days	+++†
Tench 4	2 weeks	−
Tench 5	5 days	++
Perch 1	2–3 weeks	−
Perch 2	1–2 days	++++
Perch 3	1–2 days	++
Perch 4	3 weeks	+†

* − indicates no effect on trypanosomes; +, less than 20 per cent of trypanosomes lysed in one hour; ++, approximately 50 per cent of trypanosomes lysed in one hour; +++, 80–90 per cent trypanosomes lysed in one hour; ++++, all trypanosomes lysed within 30 minutes.

† Agglutination of trypanosomes observed.

Likewise, No. 3, which had been in the aquarium the whole time and had retained a very high infection, began to feed voraciously when similarly isolated and had lost its infection by the seventh day.

Evidence that the loss of the trypanosome infection was a result of antibodies

produced by the fish—not the effect of temperature directly on the parasites—was obtained by serological studies of the blood of five tench and four perch after they were removed from the 20° C. tanks at intervals up to 21 days after trypanosomes had disappeared from the blood (Table 2). These fish were bled from the heart after receiving a blow on the head with a block of wood. The serum was removed from the clotted blood and tested for antibody activity in dilutions of 1:1, 1:2, and 1:4 parts of amphibian saline.

The fish producing the trypanosomes that were used as test antigens were kept at 10° C. and were the same species as the ones that supplied the serum. The trypanosome-infected blood was taken into a pipette that had been rinsed in citrated amphibian saline. A drop of this blood was mixed with two drops of the diluted serum. A comparison of the number of trypanosomes present immediately after mixing the serum and blood with the number surviving one hour later gave evidence of lyses of the trypanosomes. While the counts were being made, any agglutination of the parasites was observed.

The results of the immunological studies are summarized in Table 2. It was possible to demonstrate the production of trypanocidal antibodies by the perch and the tench. The antibodies may not destroy all the trypanosomes, and those that remain show no visible effect from the antibodies. Evidence that the antibodies are rapidly dissipated is given by the fact that no antibody activity could be demonstrated after the infection had been reduced for two to three weeks. This evidence supports earlier observations on bacterial infections in fish.[5]

These observations, in general, support those previously made on the immunology of fish and amphibians.[6, 7] The temperature at which the fish are maintained and the feeding of the fish have been demonstrated to be two factors involved. The observations also show that the production of antibodies may be controlled by the social position of the animal, particularly if strong territorial behavior develops with heavy population density of the hosts. Observations indicate that, once the behavior pattern is sufficiently established, it will be maintained in spite of the removal of the dominant animal.

* This work was carried out in the department of zoölogy, University of Cambridge, England, while the author was a Fellow of the Fund for the Advancement of Education of the Ford Foundation. He wishes to acknowledge his indebtedness to both of these institutions.

[1] M. E. Brown, "The Growth of Brown Trout, *Salmo trutta*, Linn.," *J. Exptl. Biol.*, **28**, 473–480, 1951.

[2] B. Greenberg, "Some Relations between Territory, Social Hierarchy and Leadership in the Green Sunfish, *Lepomis cyanellus*," *Physiol. Zoöl.*, **20**, 267–299, 1947.

[3] J. C. Braddock, "Some Aspects of Dominance-Subordination in the Fish, *Platypoecilus maculatus*," *Physiol. Zoöl.*, **18**, 176–195, 1945.

[4] M. Meyer-Holzapfel, "Das Territorium als Grundlage der soziales Organization bei einer Gruppe von Schwanzbandsamlern," *Rev. suisse zoöl.*, **48**, 531–536, 1941.

[5] K. A. Bisset, "The Effect of Temperature upon Antibody Production in Cold-blooded Vertebrates," *J. Pathol. Bacteriol.* **60**, 87–92, 1948.

[6] K. A. Bisset, "The Effect of Temperature on Non-specific Infections of Fish," *J. Pathol. Bacteriol.*, **58**, 251–258, 1946.

[7] J. H. Barrow, B. J. Vaughn, and A. M. Salyerds, "Host Specificity and Immunological Reactions against *Trypanosoma diemyctyli* in Amphibia," *J. Tenn. Acad. Sci.*, **28**, 190, 1952.

ECTOPIC DIFFERENTIATION OF EARS IN SECONDARY EMBRYOS OF FUNDULUS

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Introduction.—Although it was first demonstrated almost twenty years ago[1] that fragments of the early embryonic shield of *Fundulus* differentiate successfully after implantation to the extra-embryonic membrane of hosts the same age as the donors, no systematic analysis has subsequently been made of the comparative capacities for differentiation of various portions of the shield after such transplantation. Accordingly, an investigation is currently in progress for the purpose of studying on the extra-embryonic epithelium the development of fragments representing four different antero-posterior levels of the *Fundulus* shields. While final description and interpretation of the results must await completion of the histological study of the experimental embryos, inspection in the living material of the patterns of differentiation accomplished and evoked by the grafts reveals some features of sufficient significance to warrant their presentation at this time.

Experimental Procedure.—The grafts consisted of fragments obtained by dividing stage 13 or stage 14[2] shields antero-posteriorly into four parts, of approximately equal length (Fig. 1). These were implanted on the extra-embryonic membrane of hosts approximately the same age. All layers constituting the shield were included in the grafts. The hosts were raised to stage 31 or stage 32, the pre-hatching or hatching stages, respectively.

Observations.—The secondary structures identifiable in the living condition which were differentiated by the grafts or in association with them included brain, spinal cord, eye and lens, ear, heart, pericardium, notochord, segmented musculature, urinary bladder, peritoneum, and pectoral and longitudinal fins. When similar grafts, in another experimental series,[3] had been implanted into the shield rather than on extra-embryonic membrane heart, pericardium, bladder, peritoneum and longitudinal fin were not differentiated, and pectoral fin was formed with far less frequency. Furthermore, as far as the components of the secondary aggregates which can be identified in the live material are concerned, the variety of structures differentiated as a result of implantation of grafts derived from any specific level seemed considerably wider after transplantation to extra-embryonic than to embryonic regions; this was particularly evident for grafts from the posterior parts of the shield.

Of equal interest with the specific content of the secondary masses is the arrangement of their constituent organs within them. In a number of cases, following the extra-embryonic implantation of the fragments designated 3 and 4 in Figure 1, the secondary structures were organized to form entities resembling trunk and tail, in which the principal axial structures exhibited their typical topographic relationships to one another. These represented secondary embryos which, though smaller than their primary counterparts, were sometimes of remarkable quality (Figs. 2 and 3).

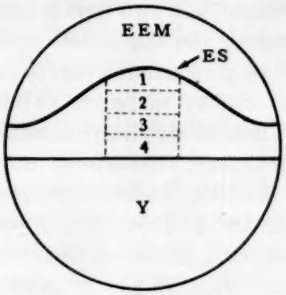

FIG. 1.—Scheme of operations, indicating the sources of the fragments (1, 2, 3, 4) utilized for grafts. Dorsal view of shield in intact *Fundulus* egg. Regions 1, 2, 3, and 4 indicate approximate regions of prospective forebrain, midbrain, hindbrain, and spinal cord, respectively, at stage 13, the midgastrula stage. *ES*, embryonic shield; *EEM*, extra-embryonic membrane; *Y*, yolk.

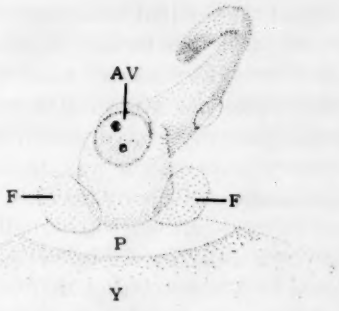

FIG. 2.—Anterior view of a secondary embryo induced by the implantation of a fragment 4 from a stage 13 shield on the extra-embryonic membrane of a stage 13 host, drawn at stage 30 to 31. An auditory vesicle (*AV*) containing two otoliths has differentiated dorsal to the neural axis and considerably posterior to the level of attachment of the paired pectoral fins (*F*). *P*, pericardium; *Y*, yolk. Magnification, ×55.

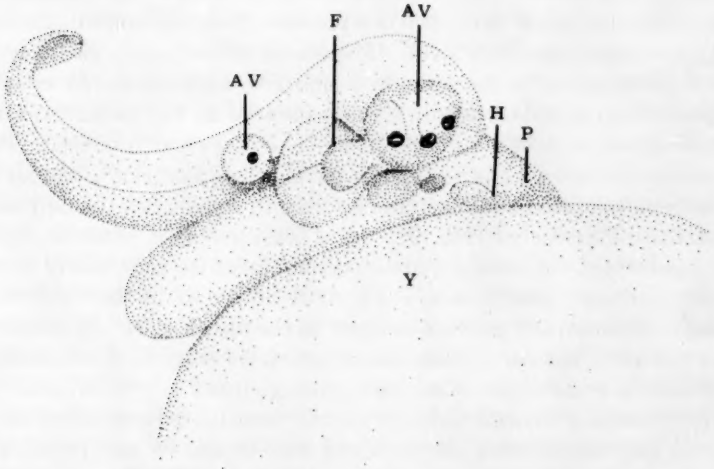

FIG. 3.—Lateral view of a secondary embryo induced by the implantation of a fragment from a stage 13 shield on the extra-embryonic membrane of a stage 13 host, drawn at stage 31. The anterior end of the induced embryo is shown at the right of the figure. In this embryo paired auditory vesicles with differentiated canals have formed anterior to the fins; the right member of the pair, containing three otoliths, is seen at the right of the drawing. Another smaller and less highly differentiated auditory vesicle, unpaired and containing only a single otolith, located posterior to the fin in the embryo, is farther to the left of the figure. *AV*, auditory vesicle; *F*, fin; *H*, heart; *P*, pericardium. Magnification, ×55.

The tails of the induced embryos were rarely straight, and two were bifid. The anteriormost structures formed in embryos induced by the implantation of fragment 3 or fragment 4 consisted most often of heart or pericardium, ear, or pectoral fin. Pericardium was often differentiated in the absence of heart. The heart, when present, was rhythmically contractile but failed to establish circulation within the secondary embryos; it was, in fact, a characteristic attribute of the extra-embryonic grafts and their associated structures that they lacked vascularization in almost all cases, although the vitelline vessels of the hosts coursed adjacent to and sometimes immediately below them. The pectoral fins were unique among the induced structures in that they were frequently equal in size to those of the host, and in some cases they were functional. In several induced embryos they were reduplicated.

The auditory vesicles differentiated by the secondary embryos were in most cases small and retarded in development as compared with the primary ones, failing to differentiate canals but containing in some cases the two otoliths characteristic of the early *Fundulus* ear, in other cases only a single one. The production of ear rudiments is appropriate for embryos induced by the implantation of fragment 3, which includes the prospective hindbrain region, and is not wholly unexpected at the anterior limits of those produced by the implantation of fragment 4, which presumably undergoes some regulation when isolated on extra-embryonic epithelium and which in any case borders anteriorly on the prospective hindbrain region. Especially noteworthy, therefore, is the position in the induced embryos of the ear primordia, which developed in a number of cases at some distance caudad of their typical antero-posterior level. These were located posterior to the pectoral fins, in the trunk region of the secondary embryos, adjacent to notochord and to what appears to be spinal cord, with somites anterior as well as posterior to them. Their development in mid-notochord levels is of particular interest in *Fundulus*, where the chorda normally has its anterior termination posterior to the ear level.

The ectopically located auditory vesicles were in some cases the only secondary ear rudiments differentiated (Fig. 2); in at least one case, however, such a primordium was located well posterior to the pectoral fin on the right side of the embryo, while other secondary paired ears, much more advanced in their differentiation, were found in their normal position anterior to the fins (Fig. 3). A case somewhat similar to the latter has been illustrated at an earlier stage in development for an induced *Fundulus* embryo described some time ago (see Fig. 9 of an earlier paper[1]); here a single medially located auditory vesicle was situated anterior to the secondary pectoral fins, and another ear rudiment was produced just posterior to the left secondary pectoral fin.

Discussion.—The fact that the ear, which normally develops at a single specific antero-posterior level of the embryonic axis, can also differentiate at an appreciably more posterior level, in association with structures not usually found in close topographical relationships with it, is of considerable interest. Otocysts have frequently differentiated atypically in the amphibian as a result of the use of abnormal inductors[4–7]; Chuang[5] and Toivonen[6] reported an unexpectedly high frequency of otocysts at trunk levels after implantation of abnormal inductors into the blastocoele, but this has been accounted for as possibly a result of interaction of regional influences between host and graft.[8] The factors responsible for the atyp-

ical location of the auditory vesicles in *Fundulus* must be sought in the secondary embryos themselves rather than in the relationships of these to the primary embryo, for, as previously emphasized[1] and as confirmed by the character of the structures developed in the present series, the differentiation of parts located remote from the hosts on the *Fundulus* extra-embryonic membrane is not subject to influence from the primary embryo.

Chuang[5] also studied the differentiation of explants in which fresh *Triton* liver has been combined with prospective ectoderm. At least one of these gave rise to auditory vesicles, though it consisted otherwise only of nonbrainlike nerve tissue, chorda, muscle, and connective tissue. Yamada and Takata[7] reported otocysts to have differentiated, following the implantation of mouse kidney in isolated ectoderm, in explants consisting largely of spino-caudal structures (cf. their Table 3, which lists 7 out of 32 such explants as containing ear vesicle and only 4 as containing brain). As a matter of fact, the occasional differentiation of ears in fish isolates containing primarily trunk and tail structures has also been reported for the trout,[9] in an experimental series in which prospective ectoderm of trout had been combined with posterior archenteron roof (trunk organizer) on the yolk-sac epithelium of older trout fry.

The significance of ear formation under the influence of living or abnormal inductors in irregularly shaped explants or isolates containing primarily spino-caudal structures is hardly to be minimized. Ectopic ear differentiation in such highly organized secondary embryos as discussed here for *Fundulus* demonstrates, however, even more dramatically that there may be mechanisms involved in the production of auditory vesicles which have hitherto escaped detection. In the first place, the fact that ear rudiments predominate as the ectopic structures suggests that these organs may be more readily induced than are other organs by agents not typically active in their differentiation, a contingency which may be related to the fact that differentiation of the ear, at least as exemplified by that of the amphibian, has been demonstrated to be affected by a greater number of factors than is that of most other organs so far investigated.[10] Interesting, however, as the agents responsible for governing the differentiation of ears per se may be, the present results are of wider significance: since they demonstrate the operation of factors functioning at various points along the primary axis, they may open a way to investigate the differentiation of a highly localized structure as related to the organization of the embryonic axis as a whole. While their full significance remains to be evaluated it may be stated now that the reported data are extremely difficult to reconcile with the concept that a single inducing system, diminishing quantitatively as a gradient along the axis, could be responsible for releasing the diversity of form which characterizes the vertebrate embryo.

Summary.—The development of fragments representing four antero-posterior levels of the embryonic shield of *Fundulus* has been studied after their implantation to extra-embryonic regions of hosts of approximately the same age as the donors. The variety of structures formed in association with grafts of any particular level is greater after implantation to extra-embryonic than after implantation to embryonic regions. Of particular interest, however, is the arrangement of structures differentiated and induced by the grafts. The fragments representing the two posterior quarters of the shield often induce highly organized embryos containing,

besides a number of trunk and caudal structure, auditory vesicles which are frequently located posterior rather than anterior to the secondary pectoral fins. The fact that the ear, which normally develops at a single specific antero-posterior level of the embryonic axis, can also differentiate more caudally in organized secondary embryos, suggests that there may be mechanisms involved in ear production which have hitherto escaped analysis and which have a fundamental significance with relation to the factors governing the establishment of the primary embryonic axis as a whole.

[1] J. M. Oppenheimer, *J. Exptl. Zool.*, **72**, 409–437, 1936.

[2] J. M. Oppenheimer, *Anat. Record*, **68**, 1–15, 1938.

[3] J. M. Oppenheimer, *J. Exptl. Zool.*, **128**, 525–559, 1955.

[4] J. Holtfreter, *Arch. Entwicklungsmech. Organ.*, **132**, 307–383, 1934; S. Toivonen, *Ann. Zool. Soc. Zool. Botan. Fennicae Vanamo*, **11**, 1–28, 1945, and *Arch. Soc. Zool. Botan. Fennicae Vanamo*, **6**, 66–71, 1951.

[5] H.-H. Chuang, *Arch. Entwicklungsmech. Organ.*, **139**, 556–638, 1939.

[6] S. Tovonen, *Ann. Acad. Sci., Fennicae, A*, **55**, 1–150, 1940.

[7] T. Yamada and K. Takata, *J. Exptl. Zool.*, **128**, 291–331, 1955.

[8] J. Holtfreter and V. Hamburger, in B. H. Willier, P. A. Weiss, and V. Hamburger (eds.), *Analysis of Development* (Philadelphia: W. B. Saunders Co., 1955), Sec. VI, chap. i.

[9] R. M. Eakin, *Arch. Entwicklungsmech. Organ*, **139**, 274–281, 1939.

[10] J. Holtfreter, *Arch. Entwicklungsmech. Organ.*, **127**, 619–776, 1933; R. G. Harrison, *Anat. Record*, **64** (suppl. 1), 38–39, 1935; C. L. Yntema, *J. Exptl. Zool.*, **113**, 211–244, 1950; C. L. Yntema, in B. H. Willier, P. A. Weiss, and V. Hamburger (eds.), *Analysis of Development* (Philadelphia: W. B. Saunders Co., 1955), Sec. VII, chap. iii.

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